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ANATOMICAL AND MORPHOLOGICAL STUDIES IN THE CHENOPODIACEAE

II. VASCULARIZATION OF THE SEEDLING

By T. Bisalputra*

[*Manuscript received December* 6, 1960]

Summary

An account is presented of the development of the vascular system of seedlings of *Atriplex* L., *Bassia* All., *Kochia* Roth, *Malacocera* R. H. Anderson, *Salicornia* (Tourn.) L., and *Arthrocnemum* Moq. and the anatomy of the cotyledons is described.

The procambial strands of the cotyledons are continuous with those of the hypocotyl–radicle axis but the maturation of the first protoxylem elements occurs independently in each system and eventually they come into contact side by side. There is a certain amount of intrusion by each trace into the tissues of the other.

The extent of the transition region between the endarch protoxylem of the cotyledon and the exarch condition of the root as well as the extent of the pith within the hypocotyl and the position of initiation of maturation of the protoxylem elements seems to be determined by the manner in which the hypocotyl elongates. Almost invariably, seedlings of *Atriplex, Bassia, Kochia,* and *Malacocera* which show pronounced elongation in the upper hypocotyl have a long pith and the transition region begins low down and is extensive. Seedlings of *Salicornia* and *Arthrocnemum,* on the other hand, have a short pith and the transition region and the initiation of the first protoxylem elements occur at a high level within the hypocotyl. The seedlings of this latter group have a very pronounced elongation in the lower part of the hypocotyl.

There is no intermediate condition between the vascular system of the epicotyl which has endarch protoxylem and the root with the exarch condition, but connexion between the two is established simply by their coming into contact side by side; this occurs in the cotyledon–radicle transition zone.

It seems, in the light of this developmental study, that initially there are three separate systems of vascular tissues within the seedling, those of the epicotyl, the cotyledon, and the radicle, but further evidence is needed before a definite conclusion can be made and this could be obtained by an embryological study of the group.

I. Introduction

Our knowledge of the anatomy of the seedlings of plants was established by the intensive studies of plant anatomists and morphologists who were inspired mainly by the recapitulation concept during the period covering the latter part of last century and the first two decades of this century. The work of Dangeard (1889), van Tieghem (1871, 1891), Fron (1899), Hill and de Fraine (1908, 1909*a*, 1909*b*, 1910, 1912), Compton (1912*a*, 1912*b*), etc. was mainly descriptive and the emphasis was on the root–shoot transition of the vascular system. They have, however, added much to our knowledge of the orderly sequence of vascular development of the seedlings. A few

* Department of Botany, University of New England, Armidale, N.S.W.

theories such as those of Thomas (1907) and Compton (1912*b*) were put forward and these again were concerned primarily with the transition of the vascular system from root to shoot, especially of those seedlings whose germination is epigeal.

As far as the anatomy of the seedling of the Chenopodiaceae is concerned, Fron (1899) in particular and Hill and de Fraine (1912) have treated the subject quite extensively with emphasis on the root–shoot transition of the following genera: *Atriplex* L., *Salsola* L., *Suaeda* Forsk., *Beta* (Tourn.) L., *Spinacia* (Tourn.) L., *Salicornia* (Tourn.) L., *Chenopodium* (Tourn.) L., *Blitum* (Tourn.) L., *Obione* Gaertn., *Haloxylon* Bunge, *Anabasis* L., *Kochia* Roth, *Rhagodia* R.Br., *Corispermum* B. Juss. ex L., *Roubieva* Moq., and *Camphorosma* L. These works and others in the past have dealt also with comparative studies of older seedlings. The present study, on the other hand, is directed at the development of the entire vascular system particularly the differentiation of the proto- and metaxylem elements, and the manner of connexion between epicotyl vascular traces and the hypocotyl–cotyledon system.

II. Material and Method

(*a*) *Material*

Seedlings of the following Australian members of the family Chenopodiaceae were investigated: *Atriplex leptocarpa* F. Muell., *A. fissivalvis* F. Muell., *A. semibaccata* R.Br., *A. nummularia* Lindl., *A. vesicaria* Hew. ex Benth., *A. spongiosa* F. Muell., *A. intermedia* R. H. Anderson, *A. inflata* F. Muell., *Bassia divaricata* (R.Br.) F. Muell., *B. brachyptera* (F. Muell.) R. H. Anderson, *B. lanicuspis* F. Muell., *B. birchii* F. Muell., *B. ventricosa* J. M. Black, *Kochia triptera* Benth., *K. georgei* Diels, *K. sedifolia* F. Muell., *K. astrotricha* L. Johnson, *K. brevifolia* R.Br., *K. aphylla* R.Br., *K. pyramidata* Benth., *K. coronata* J. M. Black, *Malacocera tricorne* (Benth.) R. H. Anderson, *Salicornia australis* Banks & Sol., and *Arthrocnemum halocnemoides* Nees var. *pergranulatum* J. M. Black.

Seeds of *Atriplex leptocarpa*, *A. semibaccata*, *Kochia brevifolia*, *K. aphylla*, and *M. tricorne* were obtained from Mr. John Warren of the Soil Conservation Service of New South Wales at Condobolin. Seeds of *Salicornia* and *Arthrocnemum* were received from Mr. T. E. H. Aplin of the Department of Agriculture, Perth, W.A. Other seeds were collected at Fowler's Gap Research Station, 70 miles north of Broken Hill, N.S.W., in February 1959 by the author.

All seeds were germinated in an incubator at 27°C. Those of *Atriplex*, *Salicornia*, and *Arthrocnemum* germinated with ease but there was some difficulty in the other genera, which, it was found, could be overcome by removing the enclosing fruiting perianths from the seeds before incubating. Using this method, *K. pyramidata*, for example, shows an increase in germination from 5–10% to almost 95%, emergence occurring within 36 hr instead of the normal time lapse of 4–8 days.

A proportion of seedlings of the very young stages of germination was removed from the oven and fixed in formalin–acetic–alcohol. The rest were transplanted into pots and placed in the greenhouse of the Botany Department, University of New England. Older seedlings were then collected from these pots at intervals

until the stage when the first two leaves were about 3–4 mm long. These older seedlings were also fixed in F.A.A.

(*b*) *Method*

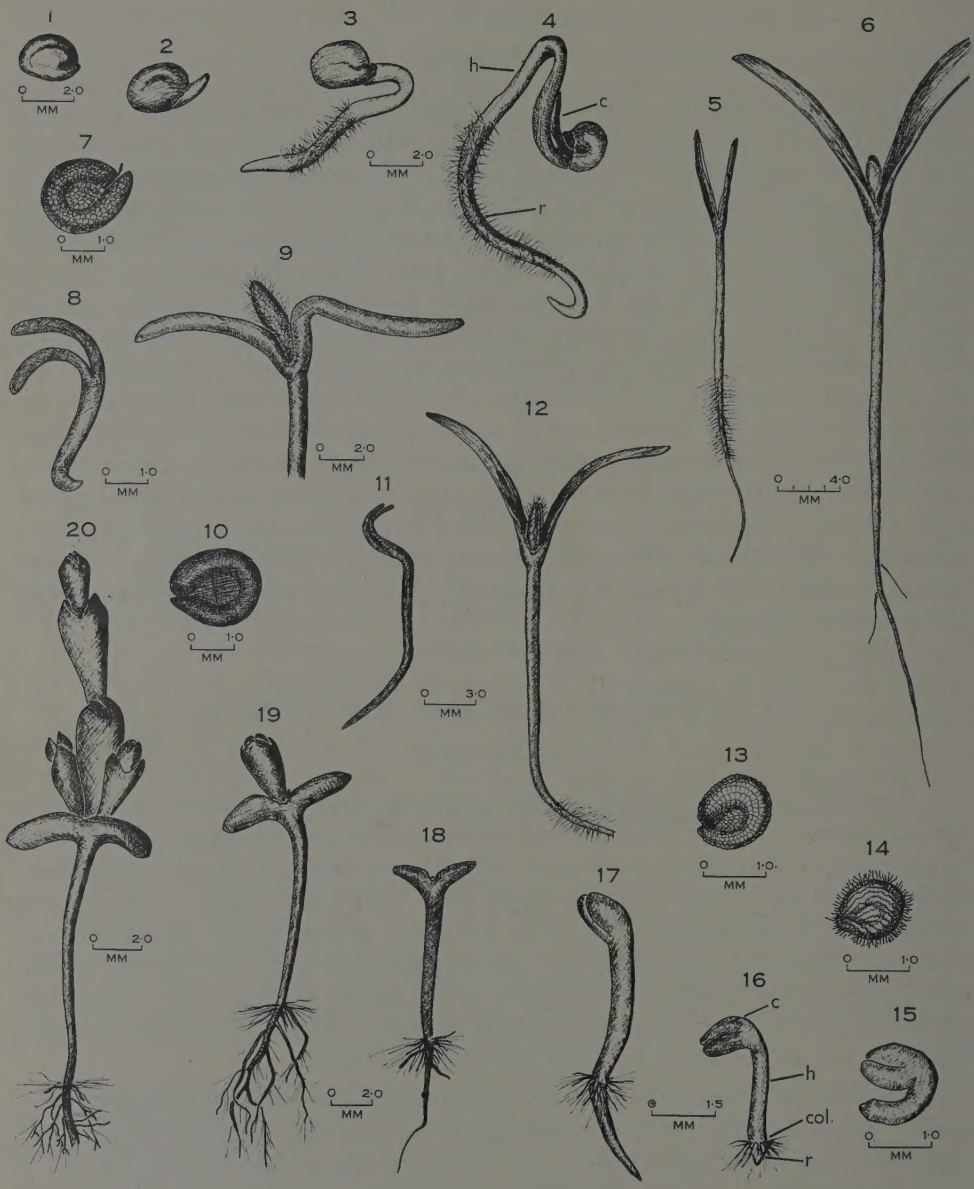
Whole seedlings were cleared in chlorallactophenol by the method employed previously by the present author (Bisalputra 1960). It was found, however, that this could be improved upon by adding a small amount of concentrated hydrochloric acid to the solution during treatment. The method is summarized as follows: Material fixed in F.A.A. was transferred to a test-tube containing solution of chlorallactophenol (1 part chloral hydrate, 1 part carbolic acid, 3 parts lactic acid), one or two drops of concentrated hydrochloric acid were then added to every 10 c.c. of solution, and the test-tube was heated in the sand bath set at 100°C for 10–20 min. Cleared specimens were mounted in welled slides and observation was made under the microscope. On the average between 25 and 30 seedlings of various stages were investigated for each species except *B. divaricata* and *B. ventricosa*, in each of which 6 seedlings were studied. This clearing method was employed in the study of the whole vascular system, particularly the differentiation of the xylem. For the anatomy and the development of the cotyledons this method was supplemented by the conventional paraffin sections, cut at 15 μ. Paradermal sections of cotyledons were cut at 10 μ and all sections were stained in safranin-fast green. A micro-projector was used in the detailed drawing of the cleared material.

III. Morphology and Anatomy of the Seedling

(*a*) *The Seed*

Seeds of plants in the family Chenopodiaceae are characterized by having a curved embryo. Ulbrich (1934) divided the family into two groups according to the arrangement of the embryo in the seed. The first and larger group is the Cyclobeae, having the embryo curved around the perisperm. This group includes all the genera investigated in the present work. In a few cases perisperm may be absent, e.g. *Salicornia*. The second and smaller group is the Spirolobeae, of which *Suaeda*, *Salsola*, and *Haloxylon* are members. They have either spiral embryos which occupy the whole seed or more or less straight or slightly curved embryos if endosperm is present. The embryos usually divide the endosperm into two halves.

Seeds of *Atriplex* species are vertical and enclosed between two opposite bracteoles. The pericarp is thin and membranous but the testa is rather leathery and shiny. The size of the seeds ranges from 1·5 to 2·5 mm in diameter and 1·0 to 1·5 mm in height. The testa is composed of two integuments and the embryo completely curves around the perisperm, which contains mainly starch. The cotyledons in a mature embryo are about half as long as the entire embryo. The outer cotyledon is, however, slightly longer than the inner one. In general there was no significant difference between seeds of the various species examined although those of *A. nummularia* and *A. vesicaria* tended to be round whereas those of other species were slightly ovate (Fig. 1).



Figs. 1–20.—Development of seedlings of *Atriplex*, *Kochia*, *Malacocera*, and *Salicornia*. Figs. 1–6—*Atriplex lindleyi*, seed and seedlings at 36 hr, 2 days, 3 days, 1 wk, and 2 wk respectively. Figs. 7–9.—*Kochia pyramidata* showing seed and seedlings at 3 days and 3 wk respectively. Figs. 10–12.—*Malacocera tricorne* showing seed and seedlings at 3 days and 3 wk respectively. Fig. 13.—Seed of *Arthrocnemum halocnemoides* var. *pergranulatum*. Figs. 14–20.—*Salicornia australis* showing seed and seedlings of 3 days, 4 days, 5 days, 1 wk, 2½ wk, and 4 wk respectively. *c*, Cotyledon; *col.*, collet; *h*, hypocotyl; *r*, radicle.

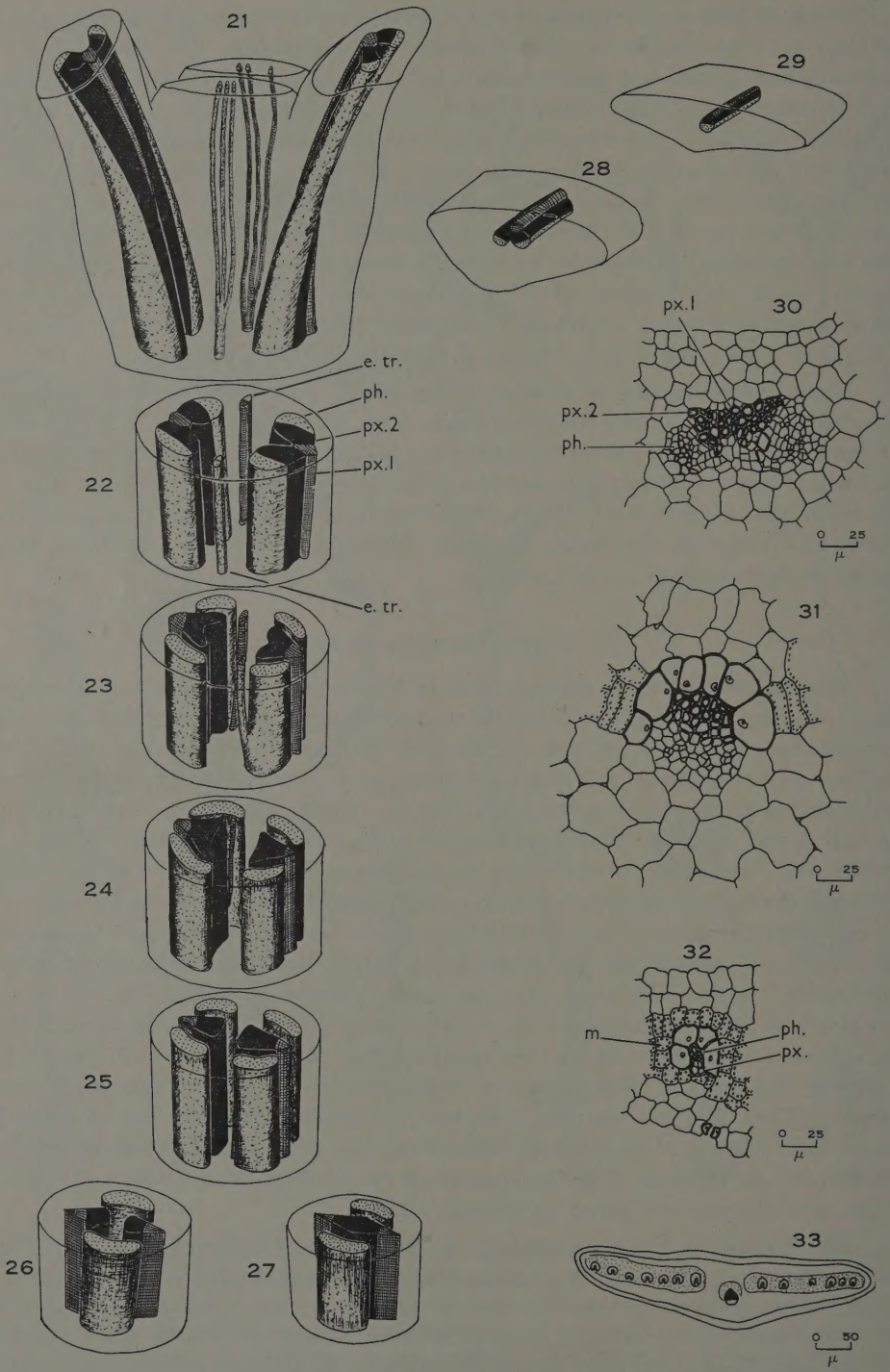
Seeds of *Bassia* and *Kochia* closely resemble each other in general appearance and also are very similar to *Atriplex* seeds described above. The *Bassia* seed is enclosed within a hard and spiny perianth tube, and most are horizontal except that of *B. lanicuspis*, which has a vertical seed. The seed of *Kochia* is contained within a perianth tube which has horizontal wings which are united, except in *K. brevifolia* where they remain separate from each other. All species used in this investigation have horizontal seeds. *Malacocera* seeds (Fig. 10) resemble those of *Kochia* and *Bassia* in every respect but they are enclosed inside a fruiting perianth which has three rather hard and spreading appendages. In all cases, seeds of these three genera have two layers of testa which correspond to the integuments, the outer being much thicker and sometimes becoming wrinkled or granulated (*K. pyramidata*, Fig. 7). The size of the seeds varies from 1·5 to 3·0 mm in diameter in these genera.

Seeds of the articulated members of the family, *Salicornia* and *Arthrocnemum*, are much smaller than those described above (Figs. 13 and 14). They average about 1·0 mm in diameter and are scarcely 0·5 mm in height; the pericarp is membranous; the testa of *Arthrocnemum halocnemoides* is horny and granulated, whereas that of *Salicornia australis* is rather leathery. The embryo of *Arthrocnemum* does not curve around the perisperm and *Salicornia* seeds contain no endosperm and the embryo usually doubles itself up, i.e. is U-shaped.

(*b*) *Development of the Seedlings*

Epigeal germination is characteristic of the family Chenopodiaceae. The pattern of development of the seedlings after the emergence shows a marked contrast between that of *Atriplex*, *Bassia*, *Kochia*, and *Malacocera* considered as one group and that of *Arthrocnemum* and *Salicornia* as the other. In the first group the radicle elongates and pushes through the testa (Fig. 2). During this phase of germination the radicle and the lower hypocotyl elongate at a much faster rate than the rest of the seedling. Elongation of the upper hypocotyl and the cotyledons becomes more noticeable during the second day. The hypocotyl usually attains a length of about 1·5–2·5 cm above the ground before elongation ceases, but the cotyledons continue to grow and finally reach their maximum development during the second week after germination. It is at this stage that the epicotyl makes its appearance. It grows very slowly at first but elongates very quickly during the third week, and thenceforth the cotyledons usually persist for a few months before they finally drop off. Seedlings of *Bassia*, *Kochia*, and *Malacocera* are very similar in both development and appearance. They have more succulent cotyledons than that of *Atriplex* and in the first two or three months it is extremely difficult in some cases to distinguish species or genera from one another. The development of the seedlings of *A. lindleyi*, *K. pyramidata*, and *M. tricorne* is shown in Figures 1–12.

The development and morphology of *Salicornia* and *Arthrocnemum* show differences from the previous group. Emergence occurs as the result of the rapid elongation of the lower hypocotyl while the radicle remains very short and stumpy (Fig. 16) and at the same time root hairs develop at the collet region. The radicle does not elongate appreciably until after the fourth day whereas the growth in the hypocotyl is slowed down markedly (Figs. 16–20). The hypocotyl attains a maximum length



[*For caption see opposite page*]

of about 0·5 cm above the ground, purely as the result of the elongation of its lower part, at or about the end of the first week. Up to this stage the cotyledons develop very slowly and they continue to do so until they reach their maximum size about the third week after germination. The two cotyledons are very succulent and are fused together at the base to form a distinct cotyledonary tube. The epicotyl, with decussate leaf arrangement, appears and grows very slowly and in general does not attain a height of 2 cm until after 5–6 weeks' growth.

(c) *Vascularization of the Seedling*

Examination of *Atriplex*, *Bassia*, *Kochia*, and *Malacocera* seedlings showed that their vascular systems are similar. Figures 21–29 were based on the study of transverse sections and cleared specimens (Plate 1) of the four genera. They are intended to show the general pattern of the vascular transition of the seedling as well as the connexion between the vascular system of the epicotyl and that of the hypocotyl–root system. This was found to be in agreement with previous work reported by Fron (1899) and Hill and de Fraine (1912). Fron (1899) pointed out that the vascular system of the *A. hastata* seedling can be regarded as typical of that genus, and he also concluded that the transition from the root system to the endarch condition of the stem always took place in the hypocotyl but at various levels. It occurred at the lower part of the hypocotyl in *Atriplex*, *Salsola*, and *Suaeda*, but at the higher level just below the cotyledonary tube in *Beta* and *Spinacia*, and at the intermediate level in *Chenopodium* and *Blitum*. Later work by Artschwager (1926) confirmed the abrupt change in the transition of the vascular system of *Beta*.

(i) *The Procambium.*—The study of the development of the procambial strands and the differentiation of the first protoxylem elements was done by using the clearing method, but with a little help from microtome sections. It was found that procambial strands could be detected readily in cleared specimens since they appeared as continuous strands of elongate cells in contrast to the shorter surrounding cells of the cortex and the pith (Plate 3).

Esau (1954) in reviewing the process of differentiation of the primary vascular system in plants stated that there is a continuity between the procambium of the hypocotyl–root system and that of the cotyledons and this is in agreement with the observations made on the pre-germination embryos in the present work. Miller and Wetmore (1945) show that in the *Phlox*, the procambium of the hypocotyl–root axis extends into the cotyledons acropetally. The present work shows that the entire

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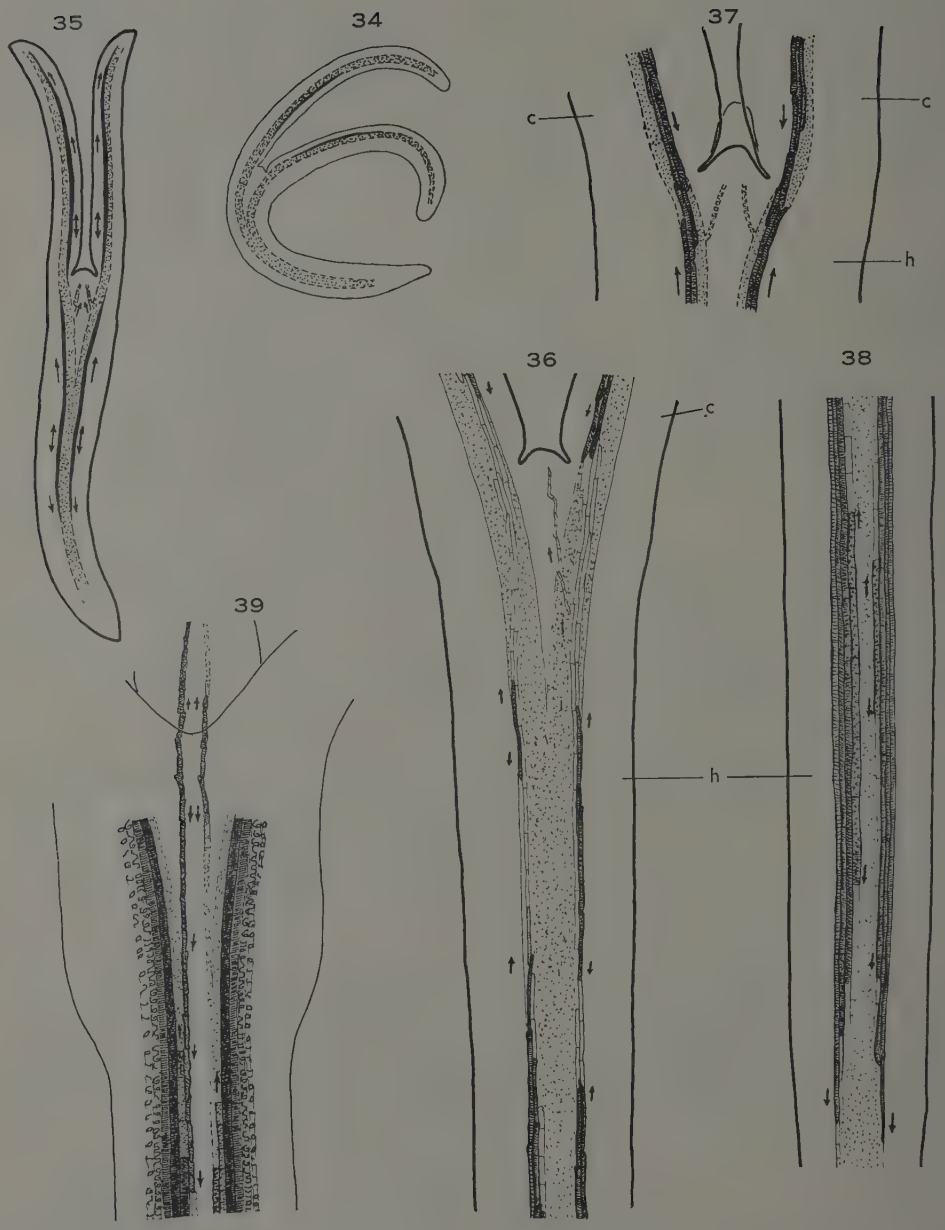
Figs. 21-29.—Vascular system of seedlings of *Atriplex*, *Bassia*, *Kochia*, and *Malacocera*, showing the transition of vascular condition of the root to those of cotyledons, and the connexion between the epicotyl traces and the root–radicle vascular system. Figs. 28–29.—The median vascular bundle at the basal and distal ends of the cotyledon respectively. Figs. 30–33.—Transverse section of the cotyledon of *A. lindleyi* showing the median bundle at the basal and distal portions of the cotyledon as well as the lateral vein of same, and also the distribution of tissues. *e. tr.*, Epicotyl traces; *m*, central mesophyll; *ph.*, phloem; *px.*, protoxylem; *px.*1, first-formed protoxylem; *px.*2, younger protoxylem.



Figs. 34–39.—Maturation of proto- and metaxylem elements. Fig. 34.—*Atriplex lindleyi* showing the procambium of the embryo prior to germination. Fig. 35.—General diagram showing the direction of differentiation of protoxylem elements. Figs. 36–39.—*A. lindleyi* differentiation of proto- and metaxylem elements of cotyledon, hypocotyl, and epicotyl vascular systems. Arrows show direction of differentiation. *c*, Cotyledon; *h*, hypocotyl.

procambial strands of both the cotyledons and the hypocotyl–radicle axis had already been laid down before germination (Fig. 34). At the apical end of the radicle the procambial strand can be observed about 10–15 cells behind the root apex. This forms a solid core extending upward into the lower hypocotyl. In *Salicornia* and *Arthrocnemum* this condition remains unchanged until a short distance below the epicotyl where the pith makes its appearance and the procambial strands on either side of the pith extend into the cotyledons (Plate 3). The pith makes its appearance much lower down in the seedlings of other genera, and may originate half way down the hypocotyl. In effect, the level of transition in these genera is much lower than that in *Salicornia* and *Arthrocnemum* (Fig. 35, Plate 1, Plate 4, Figs. 1 and 2). As the seedling begins to elongate after germination has begun, the procambial strands in the root extend acropetally and keep up with the initial rapid elongation of the root. In older seedlings where the elongation is more pronounced in the upper hypocotyl, the pith now extends a considerable distance below the cotyledonary tube. On the other hand, the pith of *Salicornia* and *Arthrocnemum* is short and confined to a short distance within or immediately below the cotyledonary tube (Plate 1, Fig. 2, and Plate 3). This is because the elongation of these seedlings occurs mainly in the lower part of the hypocotyl.

In the mature embryo, there is a single and rather flattened procambial strand at the base of each cotyledon (Figs. 42, 46, and Plate 2, Fig. 1). The trace, however, becomes narrower and smaller as it passes out toward the tip of the cotyledon (Figs. 41, 45). Branching off from this trace is a network of procambium supplying the lamina of the cotyledon. Therefore, the vascular pattern of the cotyledon is laid down before germination occurs (Plate 2). In a few embryos the first protoxylem elements were found already differentiated in the more mature part of the cotyledon (Plate 2, Fig. 2).

The procambial strands of the epicotyl cannot be detected in the embryo stage by the clearing method, but as soon as the first and second leaf primordia appear at the flank of the epicotyl apex these can be seen extending acropetally from the two procambial strands of the hypocotyl–root axis (Fig. 35).

(ii) *Maturation of the Protoxylem and Metaxylem.*—Within the two parts of the seedling, the cotyledon and the hypocotyl–root axis, maturation of protoxylem occurs independently but they eventually join up with one another and result in a continuous strand of vascular tissue running from the cotyledon at one end to the root at the other end. In most circumstances the protoxylem in the cotyledon is the first to mature. As mentioned above, the whole procambial system of the cotyledon was laid down in the embryonic stage and therefore the process of differentiation of the procambial cells into mature protoxylem elements takes place very rapidly and occurs almost simultaneously throughout the main procambial strands of the entire cotyledon. In a few cases this process of differentiation could be traced and its point of origin and the direction of maturation can be shown. These facts are illustrated in Figures 35–37. The first protoxylem element to appear in the cotyledon was observed in the embryo stage in *Atriplex* and *Bassia*. It was located within the median bundle of procambium and was about one-third of the way up from the base

of the cotyledon. Differentiation then proceeded from this point both acropetally and basipetally toward the cotyledonary node.

Soon after the first protoxylem elements began their differentiation in the cotyledon the first elements of the two opposite protoxylem strands of the hypocotyl made their appearance independently about one-third of the way between the cotyledonary tube and the collet (Fig. 35). Both were located on the cotyledonary plane and were exarch, thus the diarch condition of the vascular system of the hypocotyl was about to take shape. The differentiation of these protoxylem strands was not simultaneous since one strand usually made its appearance before the other. The differentiation of these two protoxylem traces proceeded in both directions. They differentiated towards the cotyledon protoxylem on their respective sides and finally the two traces came into contact side by side with the hypocotyl trace on the outside of the cotyledon trace (Fig. 37, Plate 3). After contact was established there was a certain amount of intrusion by each trace into the tissues of the other, and this is shown by Figure 37 and Plate 3. The important point lies in the fact that vascular connexion between the two parts of the seedling had been made. From the other end, the protoxylem elements of the hypocotyl also differentiated toward the root apex, at a rate faster than that of the elongation of the root, and eventually the diarch protoxylem of the lower hypocotyl and the root (Plate 4, Fig. 3) could be traced down to a point just behind the root apex.

The differentiation of the second, third, and fourth, and later strands of each of the diarch protoxylem points occurred successively in that order (Plate 4, Fig. 4). Each successive strand originated at approximately the same point where the first strand was formed, but as the result of rapid elongation of the upper hypocotyl during this period, the successive traces appeared to differentiate further and further away from the epicotyl. The direction of differentiation of these younger traces to the inside of the first trace followed the same pattern as that described above (Fig. 38). Plate 4, Figure 4, and Plate 5, Figure 1, show the various stages of orderly development of these traces at a point near the cotyledon tube. Frequently, instead of the orderly development of the first protoxylem trace, as described above, differentiation occurred at two or more different levels within the hypocotyl and they eventually joined with each other before the connexion with the cotyledonary trace was made (Fig. 36). Most of these earlier xylem elements had either spiral or annular thickening (Plate 3 and Plate 5, Fig. 1).

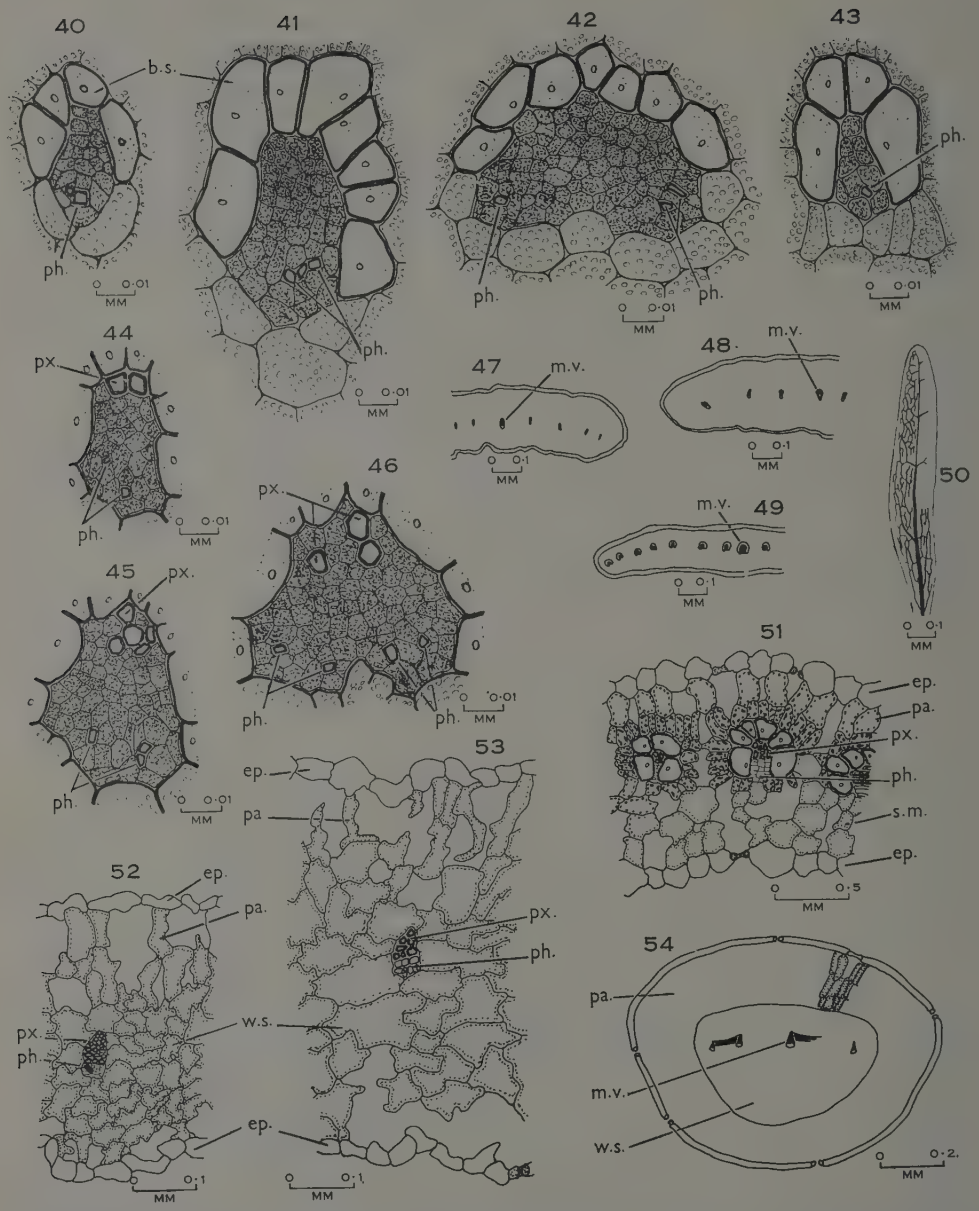
Metaxylem, on the other hand, did not differentiate until after the elongation of the hypocotyl had ceased, and consequently had a more restricted distribution than the protoxylem. Metaxylem elements first appeared about half way down the hypocotyl and their differentiation followed closely that of the protoxylem strands. In the root and the lower part of the hypocotyl they occupied the whole central portion of the axis inside the two diarch points (Plate 5, Fig. 1). In *Salicornia* and *Arthrocnemum* this condition persisted through most of the upper hypocotyl (Plate 5, Fig.1). In the other genera pith development is more pronounced and as a result the metaxylem strand was bisected at a distance well below the epicotyl (Plate 4, Figs. 1 and 2). In general, metaxylem does not intrude very far into the cotyledons. Its main connexion is rather with that of the epicotyl xylem.

(iii) *Vascular Connexion between Hypocotyl and Epicotyl.*—In the very young seedling the procambium of the epicotyl is divided into 4–5 strands. Below the cotyledonary tube, however, there are only two opposite strands located on a plane at right angles to the cotyledonary plane. Each of these two traces continues to run down the hypocotyl alongside the two hypocotyl procambia and eventually joins with them (Fig. 39). Therefore, from these two original epicotyl traces arise all the foliar traces within the shoot. The differentiation of the protoxylem of the epicotyl traces is most interesting because it leads to the eventual connexion of the vascular system of that organ with that of the hypocotyl–root system. The maturation of the protoxylem elements in the epicotyl traces begins both inside the young leaf itself and at a place just below the leaf base but they soon join and form a continuous strand differentiating acropetally toward the leaf tip and basipetally down the hypocotyl (Plate 5, Fig. 3). They do not come into contact with the protoxylem of the hypocotyl but remain separate and eventually join with the metaxylem strands which are differentiating toward the epicotyl (Fig. 39). Throughout its length each epicotyl strand has endarch protoxylem and as the connexion between the two xylem systems is made its protophloem also joins with one of the four phloem strands of the hypocotyl. These facts concerning the connexion between the vascular strands of the two systems are illustrated in Figures 21–25 and 39, and Plate 1, Plate 5, Figures 3 and 4, and Plate 6, Figure 1.

(iv) *The Vascular System and General Anatomy of the Cotyledon*

(1) *Vascularization of the Cotyledon.*—Within the procambial strands of the cotyledon the differentiation of protophloem always preceded that of the protoxylem. Figures 40–46, which are transverse sections of the median procambial trace at the tip, middle, and the basal part of the cotyledon, show this fact. As illustrated in Figures 30, 42, and 46, there are two protophloem groups in the basal part but only one in the upper part of the cotyledon. A little later the protoxylem differentiates at the upper part of the procambial strands (Figs. 44–46). There is, however, only one protoxylem group throughout the length of the cotyledon.

Protoxylem of the lateral veins matures a little distance away from the median bundle and from this point differentiation takes place both towards the median bundle and also towards the edge of the lamina. Frequently, differentiation occurs within the main bundle and proceeds outward into the lamina (Figs. 55–57). The process of maturation of the lesser veins and the formation of the islets is not unlike that in the foliage leaf of the same species. The venation of mature cotyledons of *Atriplex*, *Bassia*, *Kochia*, *Salicornia*, and *Malacocera* is shown in Figures 58–63 respectively. *Atriplex* venation shows extensive development of small veins and islets not unlike those of the leaf of the same genus (Figs. 50, 58, and 59, Plate 6, Figs. 2 and 3). Cotyledons of the other genera show a somewhat similar pattern of venation. The network of the vascular system in these genera is loosely arranged and big islets are formed by the anastomosis of the secondary and tertiary veins. Small islets are found toward the edge of the lamina but on the whole the proportion of vascular tissues per unit area is much lower than that of *Atriplex* (Plate 6, Fig. 4). In *Salicornia* and *Arthrocnemum* the venation is also rather loose and the lateral veins on the basal

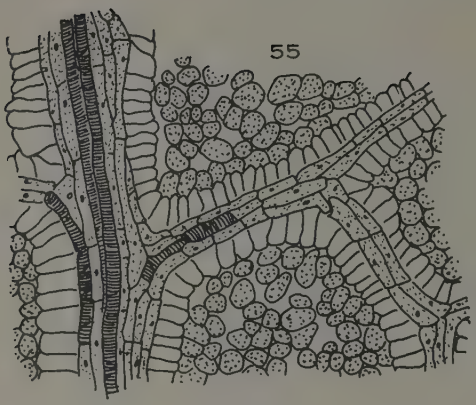


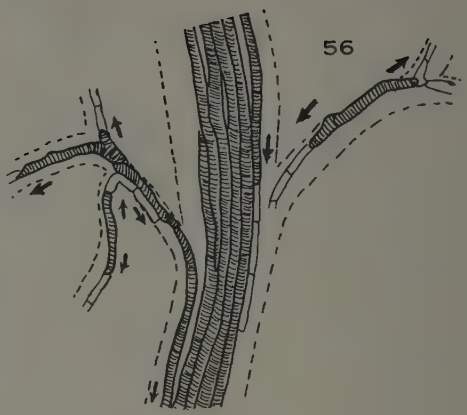
Figs. 40–54.—Transverse sections of cotyledons. Figs. 40–42.—*Atriplex lindleyi*, transverse sections of the median procambial strands showing the differentiated protophloem at the distal, middle, and basal parts of the cotyledon respectively. Fig. 43.—Transverse section of the lateral procambium of the cotyledon. Figs. 44–46.—Same as Figures 40–42 but at a later stage after protoxylem had differentiated. Figs. 47 and 52.—Transverse sections of *Bassia birchii* cotyledon. Figs. 48 and 53.—Transverse sections of the cotyledon of *Kochia pyramidata*. Figs. 49–51.—Transverse sections and venation of the cotyledon of *Atriplex lindleyi*. Fig. 54.—Transverse section of the cotyledon of *Arthrocnemum halocnemoides*. *b.s.*, Bundle sheath; *ep.*, epidermis; *m.v.*, mid vein; *pa.*, palisade mesophyll; *ph.*, protophloem; *s.m.*, spongy mesophyll; *w.s.*, water storage cells; *px.*, protoxylem.

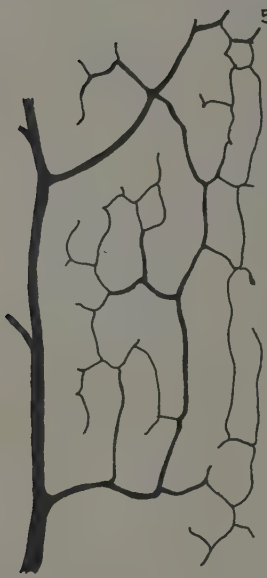
part of the cotyledons anastomose with one another within the cotyledonary tube (Plate 5, Fig. 2).

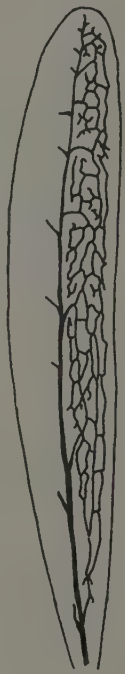
(2) *Anatomy of the Cotyledon.*—As was mentioned above, the venation of the cotyledon of *Atriplex* is not unlike that of the foliage leaf, but there are a few differences between the two organs. Firstly, the epidermis, even though it resembles that of the leaf (Black 1954; Bisalputra 1959), is devoid of hairs or trichomes of any kind. The epidermal cells have thin walls and contain no pigments of any sort. Stomata are numerous in both upper and lower epidermis and their distribution seems to be at random. Secondly, the *Atriplex* cotyledon cannot be called a perfectly isobilateral type of foliage organ (Figs. 32, 33, 49, and 51). Almost invariably the layer of cells which corresponds to the hypodermis in the leaf tends to be elongated and palisade-like. It is one cell layer thick and has enormous air spaces and the cells contain numerous chloroplasts. Underneath this layer lie the two assimilatory tissues so typical of the "Kranz" type of assimilation tissues. The inner cells form the thick-walled bundle sheath and contain a large number of chloroplasts, whereas the cells on the outside of the bundle sheath are much smaller than those in any of the tissues described previously. They are elongated and contain numerous chloroplasts and must constitute the main assimilatory tissues of the cotyledons. They tend to be more compact, with few or no air spaces between them, and they generally surround the bundle sheath completely. The layer of cells between this assimilatory tissue and the lower epidermis gives the cotyledon its succulent appearance. These cells are loosely packed and resemble spongy mesophyll to some extent. They are irregular in shape but do not branch extensively and contain a few chloroplasts. Enormous air spaces are developed within this tissue and these open to the exterior through the numerous simple stomata. It is significant to note that mechanical tissues other than xylem elements are absent altogether from the cotyledon. Rigidity of the cotyledon therefore seems to be attained through the succulence of the tissues and to a lesser degree from the vascular tissues, which, however, are not well developed in the genera other than *Atriplex*.

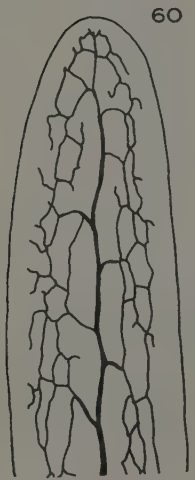
The anatomy of the cotyledons of *Bassia*, *Kochia*, and *Malacocera* will be treated together because there is practically no difference between them except for the variation in size (Figs. 47, 48, 51–53). In all three genera the epidermis is devoid of hairs and has a thin cell wall. The outer wall of the upper epidermis is often coated with a waxy material. Immediately beneath the upper epidermis there are two layers of palisade mesophyll cells with extensive air spaces. Stomata are again of a simple type and are numerous on both upper and lower epidermis. The palisade cells do not come into direct contact with the vascular tissues. The latter are surrounded by water storage parenchymatous tissues of irregular shape and closely packed together without any air spaces between them. These cells contain few chloroplasts and have well-developed vacuoles. The water storage cells occupy practically the whole space beneath the palisade tissues and towards the lower epidermis they become loosely packed and contain very large air spaces so that the last few layers resemble spongy mesophyll. The mid vein of the cotyledon is made up of a single strand of vascular tissues. There is no bundle sheath and the vascular tissues therefore come into contact directly with the water storage cells. The vascular system of the cotyledons of this

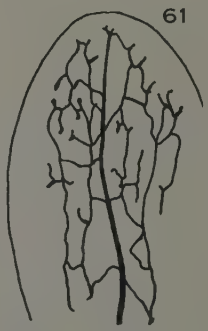


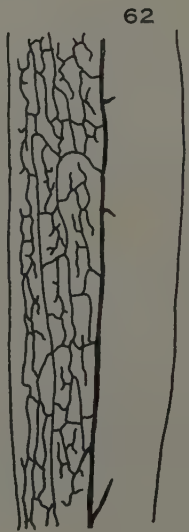


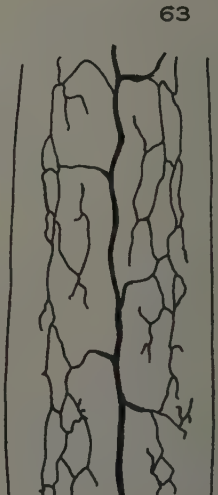












[*For caption see opposite page*]

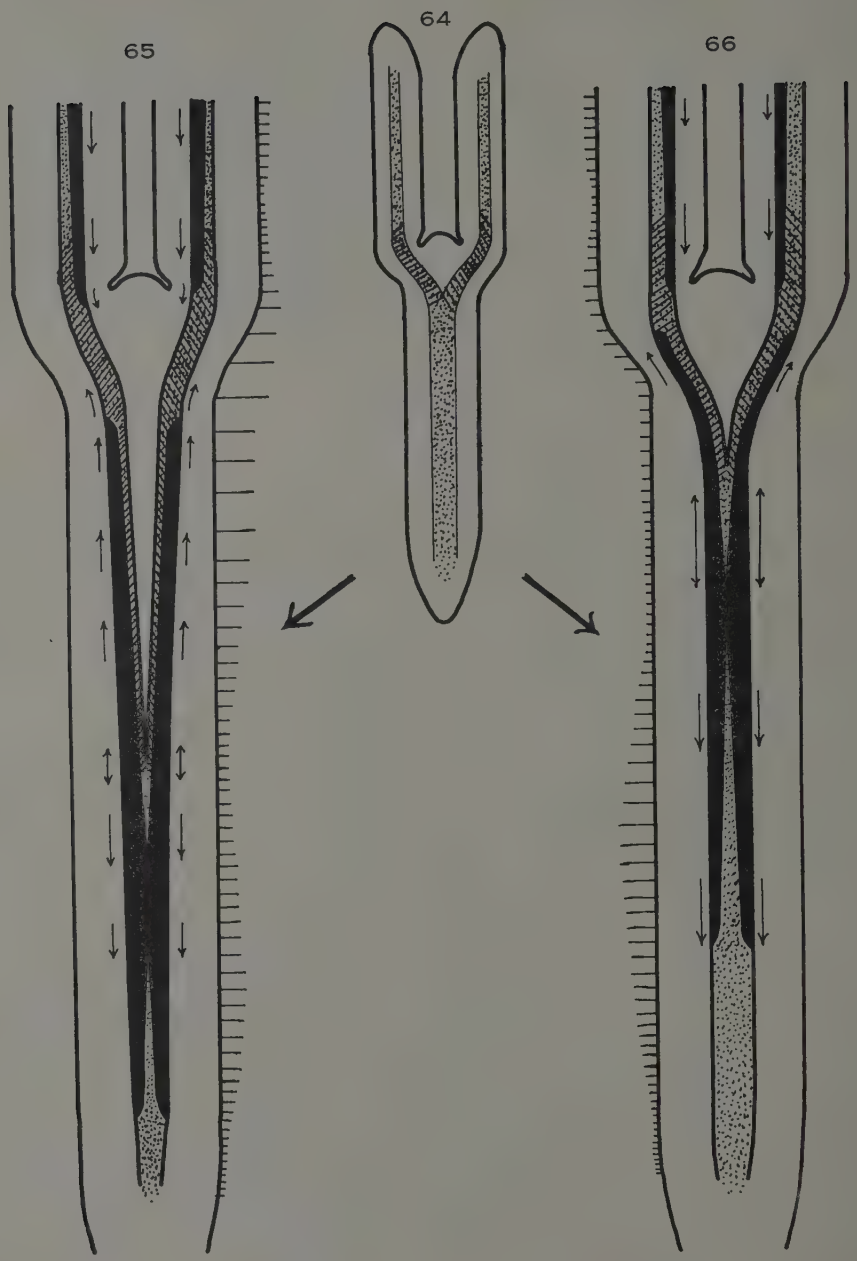
group of genera is rather poorly developed, the veins being small and widely spaced, and there is practically no development of metaxylem except for a small part at the cotyledon base.

The transverse section and the distribution of the tissues within the cotyledon of *Arthrocnemum* are shown in Figure 54. It is almost concentric in appearance. The epidermis is again without hairs and contains numerous stomata on both sides. Palisade layers form a concentric ring three to four cells thick. Air spaces are well developed within the mesophyll tissues. The vascular network lies within the water storage tissues which occupy the entire area internal to the palisade layers. The vascular system is again poorly developed and widely spaced. It should be noted here again that the mechanical tissues are again absent from both the *Kochia* group and the *Salicornia–Arthrocnemum* group.

IV. Discussion

The previous work of Fron (1899) and Hill and de Fraine (1912) on seedlings of the Chenopodiaceae, while giving much valuable information, dealt mainly with the completely differentiated vascular system and the manner in which the transition from the exarch condition to the endarch system of the cotyledon occurred. They agreed that the transition found within this family belonged to "Type 3" of van Tieghem (1891). This, in fact, implied that root of the seedling is a diarch one and the transition from the exarch to the endarch conditions took place within the hypocotyl by the splitting up of the two original radially arranged phloem strands into four. These phloem strands reorientate themselves as they pass up the hypocotyl so that they come to lie on the abaxial side of the protoxylem in the cotyledons. Compton (1912*b*) and Thoday (1939) stress the fact that there is a high degree of correlation between the slenderness of the seedling and diarch root structure on one hand, and between large diameter and tetrarch or polyarch arrangement on the other hand, and further that the size of the seedling exercises an important influence upon the whole structure of the seedling. Thoday, in explaining the process of transition, stated that each of the two apices of the seedling expresses its influence upon the structure of each respective part and that the epicotyl apex and cotyledons influence the structure of the upper part of the seedling axis and the root apex does the same at the other end. In the Chenopodiaceae seedlings, the development of the epicotyl was, almost invariably, delayed until the seedling was well vascularized. This, together with the fact that the entire procambial system of the seedling had been laid down in the embryonic stage prior to germination, would lead one to conclude that the pattern of root–cotyledon transition is determined principally by the influence of cotyledons and the root while undergoing development in the embryonic stage.

Figs. 55–63.—Venation of cotyledons. Figs. 55–57.—*Atriplex lindleyi* showing the maturation of the lateral veins and the formation of vein islets. Figs. 58–63.—Venation of mature cotyledons. Fig. 58.—*A. vesicaria*. Fig. 59.—*A. lindleyi*. Fig. 60.—*Kochia pyramidata*. Fig. 61.—*Salicornia australis*. Fig. 62.—*Bassia birchii*. Fig. 63.—*Malacocera tricorne*. Arrows show the direction of differentiation of the protoxylem elements.



Figs. 64–66.—Diagrams showing the relationship between the elongation of hypocotyl and the structure of the vascular system within the seedling. Fig. 64.—Embryo showing the procambial strands. Figs. 65–66.—Seedlings showing the two main types of elongation. Scale at the side of the seedling indicates the comparative elongation of different parts of the hypocotyl. The procambium is represented by stippling, the xylem strands by solid black, and the transition region by parallel lines. Arrows show the direction of differentiation of the protoxylem elements.

Concerning the transition between the epicotyl and the hypocotyl–root system, van Tieghem (1891) and Eames and MacDaniels (1947) postulated a process of splitting, twisting, and inversion of both phloem and xylem strands as they pass through from root to shoot. It is quite clear in this present work that this is not the case. There is no transition or intermediate condition joining the two systems, but a connexion between the two is established simply by their coming into contact side by side. Vascular strands from the epicotyl which is endarch in condition simply differentiate down the hypocotyl and join with those of the root system within the hypocotyl. The manner in which the connexion is made must be noted; almost invariably, the protoxylem of the epicotyl makes no contact with the protoxylem of the hypocotyl strands but joins with the mesarch metaxylem (Fig. 23). The phloem on the other hand joins with the hypocotyl phloem which has now come to lie to the outside of the metaxylem (Fig. 23). Evidence from the vascular differentiation points to the fact that the epicotyl and the hypocotyl–root vascular system are two separate and distinct systems and yet their connexion is established without any complicated transition.

Fron (1899) stated that the transition in the Chenopodiaceae seedling occurs at different levels in the hypocotyl. It occurs at a low level in *Atriplex*, *Salsola*, and *Suaeda*, at a high level in *Beta* and *Spinacia*, and at the intermediate level in *Chenopodium* and *Blitum*. Of the six genera investigated in this work, *Atriplex*, *Bassia*, *Kochia*, and *Malacocera* have the transition occurring at the lower level and *Salicornia* and *Arthrocnemum* at the higher level. It was found that the elongation of the hypocotyl plays an important part in determining this position. It also determines the point of the origin of differentiation of vascular tissues as well as the extent of the pith found in the young seedlings.

As a rule a seedling which has a pronounced elongation in the upper hypocotyl almost invariably has a low level of transition, a long pith extending from the plumular apex into the hypocotyl, and a low position of differentiation of first protoxylem elements. Conversely, seedlings which have pronounced elongation of the lower part of the hypocotyl, such as those of *Salicornia* and *Arthrocnemum*, tend to have a short pith, and the transition and the initiation of the process of differentiation of the vascular system seem to be confined to the upper level of the hypocotyl.

This can be explained by reference to Figures 64–66, which show these two types of growth and the relative elongation of the hypocotyl. In the first group the upper part of the hypocotyl elongates and the more mature tissues remain more or less within the lower part of the hypocotyl. It is in this region that the first mature xylem elements begin to differentiate. The transition level and the extent of the pith can be explained in the same manner. In the embryo stage the procambium forms a solid core in the hypocotyl–radicle axis but this is bifurcated below the plumule and each strand runs into the respective cotyledon. As the upper part of the hypocotyl elongates, this point of bifurcation of the procambium becomes located further and further from the plumule and therefore the pith extends more and more down the hypocotyl. Regarding the transition of the vascular system it is found that its point of origin coincides with that of the pith and at a level below this point we have a definite exarch condition of the root. On the other hand, the differentiation of tissues within the procambial strands which lie between this point and the coty-

ledons is influenced by the two adjacent systems, i.e. the cotyledonary at one end and the root at the other. Vascular tissues differentiating within this region would therefore show the intermediate condition between the two and have a mesarch xylem. As the upper hypocotyl elongates, the procambial strands lying within this area elongate accordingly, and because of this the transition system is stretched out and the condition found in *Atriplex*, *Bassia*, *Kochia*, and *Malacocera* results. Conversely, if the elongation occurs below this region the transition would remain short and high in position as found in *Salicornia* and *Arthrocnemum*.

The present investigation, therefore, shows that initially there are three systems of vascular tissues within the seedling: those of the epicotyl, the cotyledon, and the radicle. This seems to support Dangeard (1889) who stated that the vascular systems of the cotyledon and the radicle are initially discontinuous but they eventually are joined in the upper part of the hypocotyl. The extent and nature of the cotyledon–radicle transition, as the present work has shown, is dependent on the manner in which the hypocotyl elongates during the early phases of germination.

V. Acknowledgments

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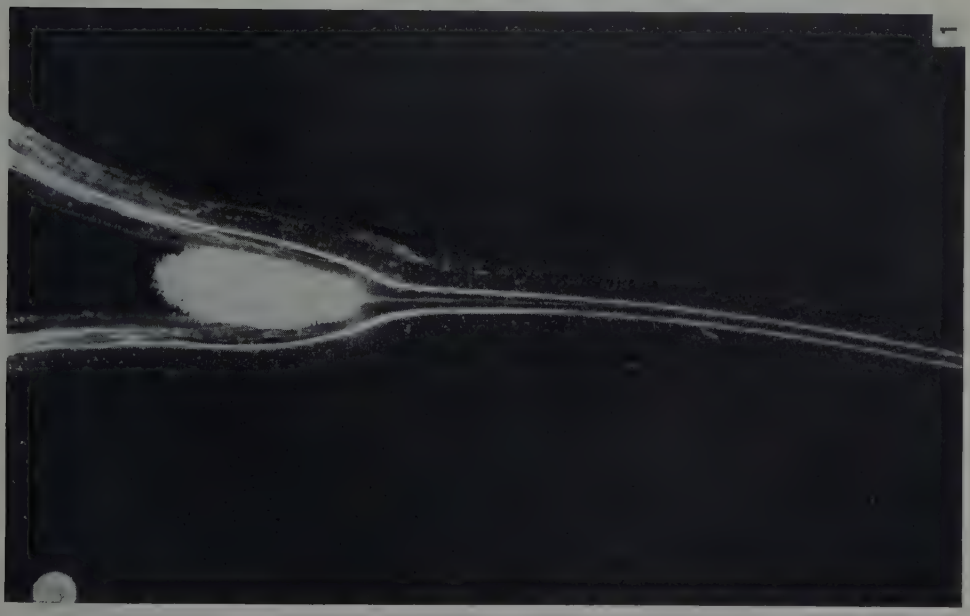

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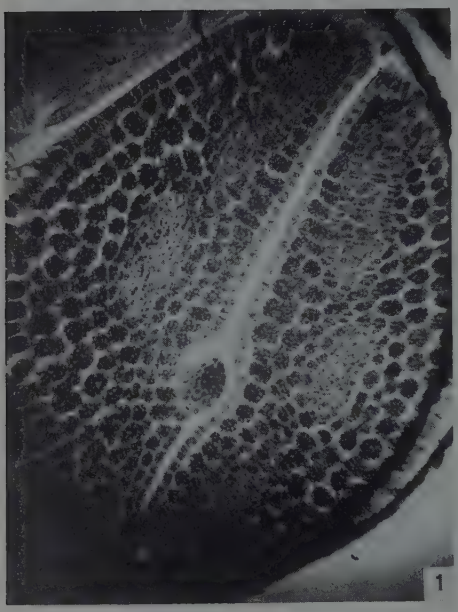

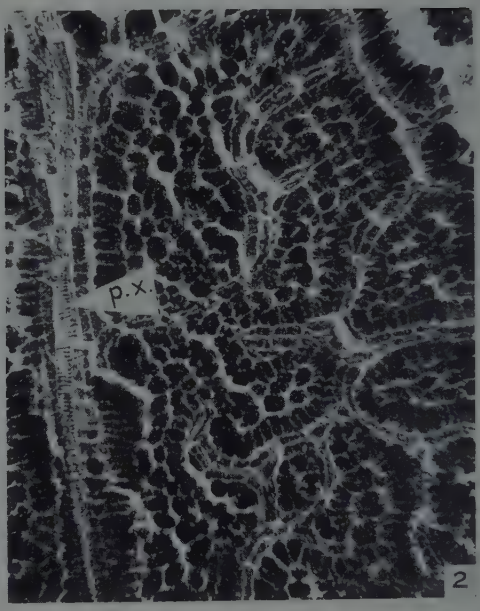

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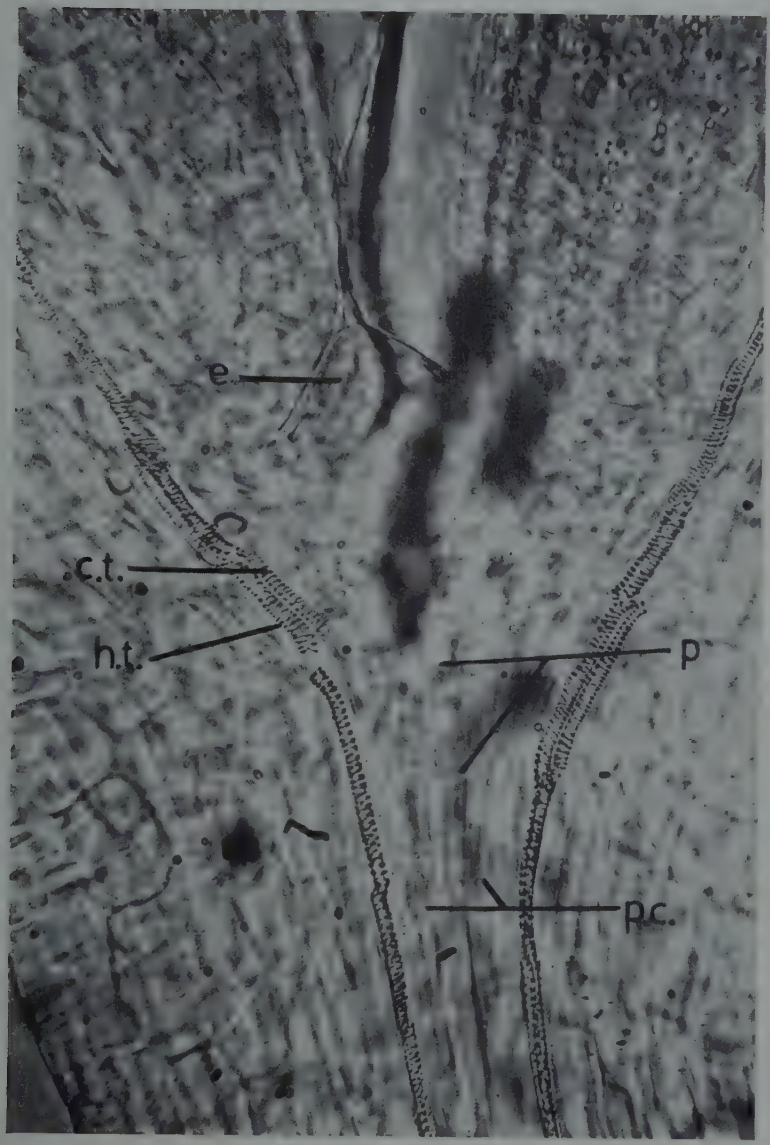

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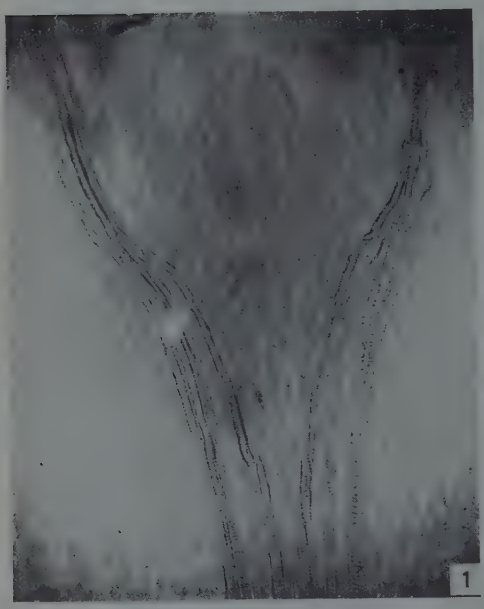

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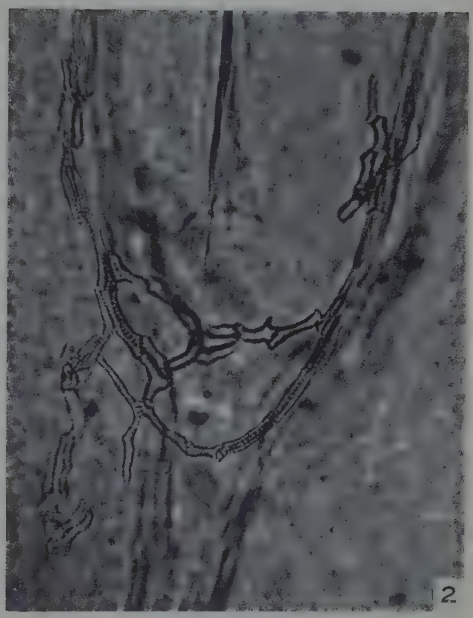

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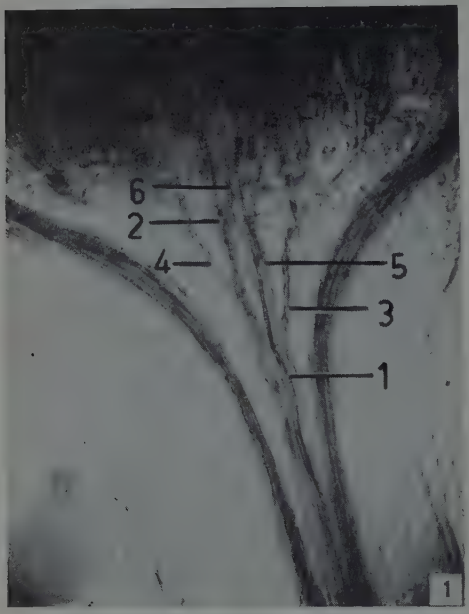

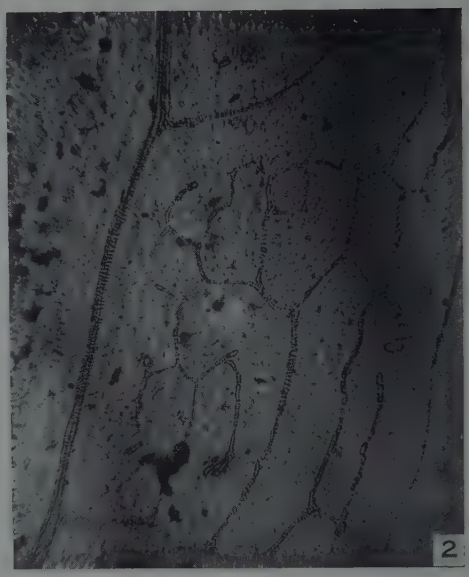

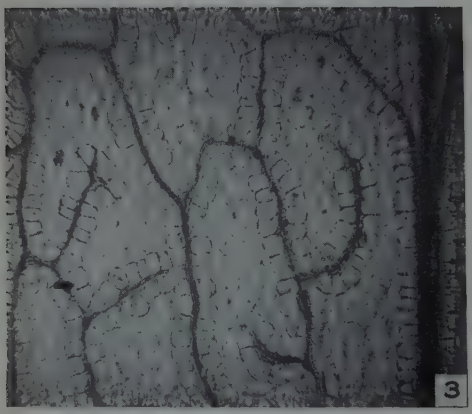

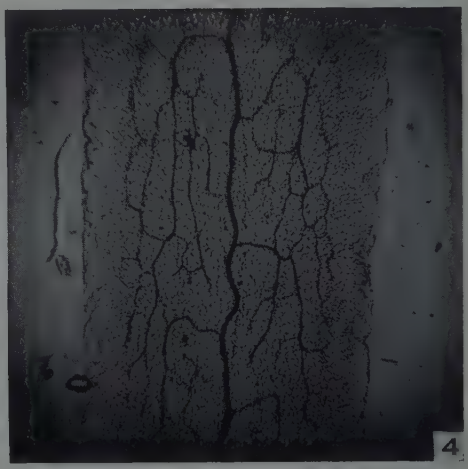

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EXPLANATION OF PLATES 1–6

PLATE 1

Fig. 1.—Cleared seedling of *Atriplex semibaccata* showing the vascular system. ×10.

Fig. 2.—Cleared seedling of *Salicornia australis* showing the entire vascular system. ×8. *col.*, Collet.

PLATE 2

Procambium of the cotyledon of a mature embryo of *Atriplex semibaccata* prior to germination.

Fig. 1.—Transverse section of the basal part of the cotyledon. ×100.

Fig. 2.—Paradermal section of the cotyledon through the median procambial strand showing the differentiated protoxylem elements. ×200. *p.x.*, Protoxylem elements.

Fig. 3.—Paradermal section at about 25 μ below the previous section. ×200.

Fig. 4.—Paradermal section showing the procambium of the lamina of the cotyledon before differentiation. ×200.

PLATE 3

Salicornia australis—cleared seedling showing the connexion between the protoxylem traces of the cotyledons and the hypocotyl. ×120. *c.t.*, Cotyledon traces; *e*, epicotyl; *h.t.*, hypocotyl traces; *p*, pith; *p.c.*, procambium.

PLATE 4

Fig. 1.—Cleared seedling of *Bassia brachyptera* showing the vascular connexion in the cotyledon–hypocotyl region. ×90.

Fig. 2.—Cleared seedling of *Bassia divaricata* showing the vascular connexion. ×75.

Fig. 3.—*Arthrocnemum halocnemoides* showing protoxylem traces in the collet region. ×100. *col.*, Collet.

Fig. 4.—*Arthrocnemum halocnemoides* showing the pattern of differentiation of the younger protoxylem elements in the hypocotyl. ×100.

PLATE 5

Cleared specimens of *Arthrocnemum halocnemoides* seedlings.

Fig. 1.—Differentiation of the metaxylem. ×100.

Fig. 2.—Anastomosis of the basal part of the lateral veins of the cotyledons. ×100.

Fig. 3.—Maturation of the protoxylem of the epicotyl. ×100. *e.t.*, Epicotyl traces.

Fig. 4.—Vascular system of an older seedling. ×9.

PLATE 6

Fig. 1.—*Bassia brachyptera* seedling showing xylem elements supplying the first six leaves of the epicotyl. 1 = protoxylem element supplying first leaf, 2 = protoxylem element supplying the second leaf, and so on. ×75.

Fig. 2.—Vascularization of the young cotyledon of *Atriplex semibaccata*. ×60.

Fig. 3.—*A. semibaccata*, vascularization of an older cotyledon. ×75.

Fig. 4.—*Malacocera tricorne*, vascularization of the cotyledon. ×20.

THE GAMETOPHYTES OF THE VICTORIAN BLECHNACEAE

I. BLECHNUM NUDUM (LABILL.) LUERSS.

By Ilma G. Stone*

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Summary

An account is given of the spore, germination, vegetative features, and reproductive organs of the gametophyte of *Blechnum nudum*.

The species is unlike other *Blechnum* spp. so far described in having a green spore, a prothallus without hairs, and an early formation of cells in a third dimension. The apical cell develops from the terminal cell of the short filament, chloroplasts are present in the rhizoids, and the mature prothallus is cordate with a slightly depressed sinus and an irregular margin. At one year the prothallus becomes elongated with a thick midrib and slightly ruffled wings.

The antheridium is elongated and has a disk or occasionally columnar-shaped basal cell, a ring cell, and an asymmetrical cap cell. Some antheridia on the cushion region become very large. Nuclear divisions in the development of the antheridium are described in detail.

The archegonium has a squat appearance, develops in the usual manner, and has a short, rather straight neck.

I. Introduction

There are two genera in Victoria belonging to the Blechnaceae, *Doodia* R.Br. (two species) and *Blechnum* L. (eight species), descriptions and the distribution of which are given by Wakefield (1955). The genus *Blechnum* is divided into two sections—the Lomaria group with dimorphic fronds and the Eublechnum group in which the sterile and fertile fronds are similar. All the Victorian species are of the Lomaria type except *B. cartilagineum* Sw., which shows the characteristics of the Eublechnum group.

Blechnum is a large genus but descriptions of the gametophytes of only six species, none of which occur in Victoria, have been published in the literature. Stokey and Atkinson (1952*a*) described the prothalli of *B. spicant* (L.) Wither and *B. buchtienii* Rosenst., and compared them with the description of *B. punctulatum* Sw. by Stubner (1882), *B. occidentale* L. by Lampa (1901), *B. brasiliense* Desv. by Jung (1927), and *B. spicant* by Karpowicz (1927) and Dopp (1927). Kachroo (1955) described *B. orientale* L. and an unknown *Blechnum* sp. Of these species only *B. brasiliense* and *B. orientale* belong to the Eublechnum group.

In order to compare the gametophytic generation of the Victorian members of the genus, spores of the eight species have been grown and the vegetative features of their gametophytes observed. The sex organs have not yet been examined in all species, but *B. nudum*, which was previously included as a form of *B. discolor* (Forst.) Keyserling, has been studied in detail, particular attention being paid to the nuclear divisions in the development of the antheridium.

* Botany School, University of Melbourne.

II. Material and Methods

Fertile fronds of *Blechnum nudum* were collected from three Victorian localities in the forest areas near Melbourne in August and October 1958 and 1959.

The fronds were washed under running water, then in several changes of sterile water, and placed between sheets of clean white paper. Spores were shed copiously and these were sown on several different media in sterile petri dishes—distilled water, boiled *Sphagnum*, mineral nutrient solutions, perlite soaked in mineral solution, and mineral agar. The mineral solutions used were Moore's (Castle 1953), and one with the following formula: KH_2PO_4, 1·0 g; $NaNO_3$, 1·0 g; $MgSO_47H_2O$, 0·3 g; $CaCl_2$, 0·1 g; NaCl, 0·1 g; $FeCl_3$, 0·01 g; distilled water, 2000 c.c.

A solid medium was made by adding 1·5% agar to this solution. Spores were also sown on unsterilized roots of *Dicksonia antarctica* Labill.

Mature prothalli were fixed in Craf 1 or F.P.A., dehydrated by the tertiary butyl alcohol–paraffin oil method and embedded in 52°C wax following the method set out by Johansen (1940). Sections were cut at 6–8 μ and stained with iron alum haematoxylon counterstained with fast green, or with safranin and fast green.

Small prothalli were fixed in Craf 1 or F.P.A., or were cleared with chloral hydrate as described by Davie (1951) to show the walls and nuclei of the developing antheridia. Various stains were tried, the most satisfactory being basic fuchsin, iron aceto–carmine, and gentian violet.

Camera lucida drawings were made of sections and of fresh and cleared material.

III. Spore Germination and Development of the Prothallus

The terminology applied to the spore is that used by Harris (1955).

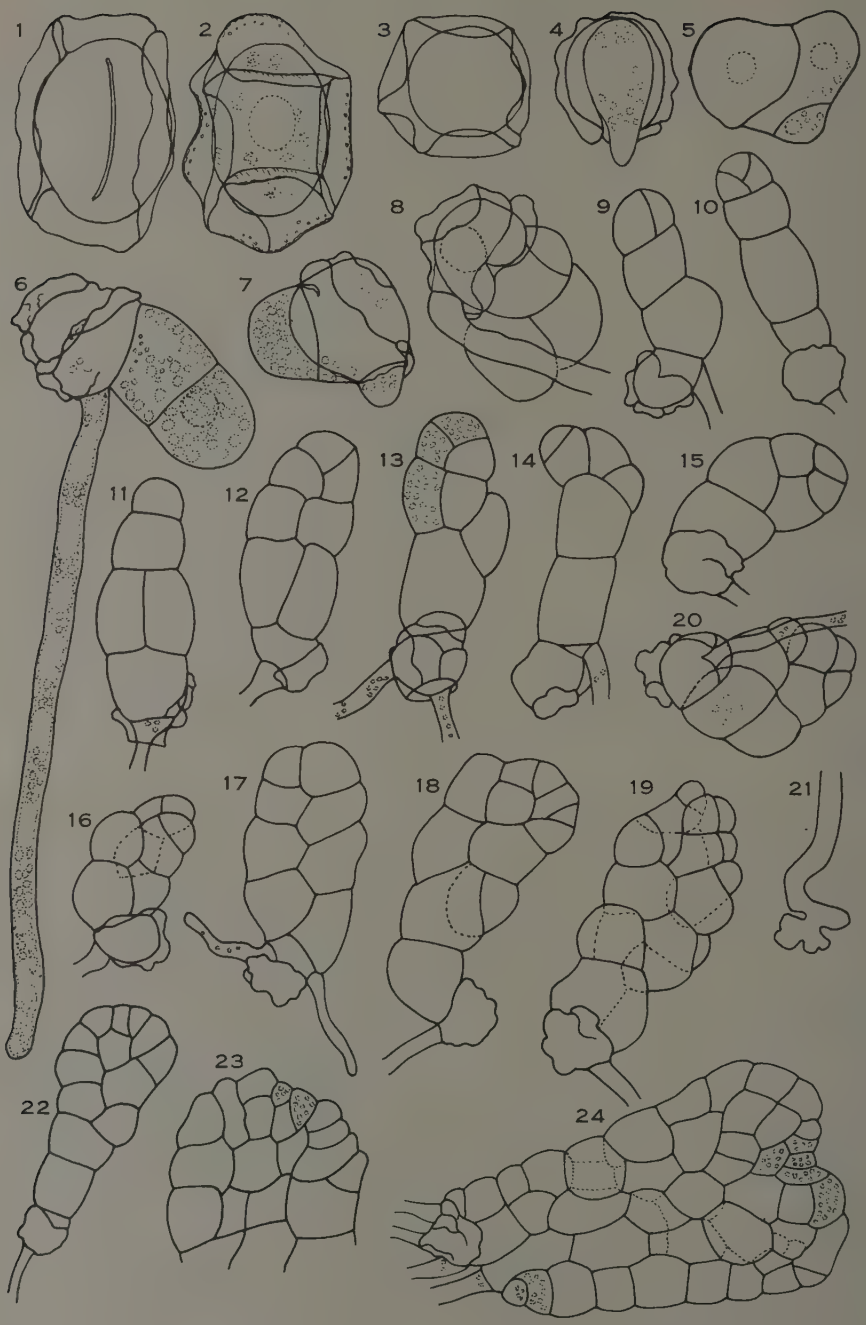
The mature spore is olive green, and contains chloroplasts and a few oil drops (Fig. 2). It is bilateral, or occasionally almost spherical, monolete, with a laesura which appears as a long, narrow inconspicuous ridge (Fig. 1). In cross section the spore is rounded (Fig. 3).

The exine is thin and has a loose winged perispore which is filmy and transparent, light brown, and smooth to faintly scabrate near the edges of the crests. The crests anastomose occasionally and vary in width up to 14 μ (Figs. 1–3).

Without the perispore, which is frequently shed, the spore usually measures 55×44 μ but there is some variation in size and in the ratio of equatorial to polar measurements.

Germination of freshly shed spores takes place in 2–4 days on all media, but viability is considerably reduced with age and germination of spores which are 3 months old is very sparse and takes 10 days.

On germination, the exine splits along the laesura, the spore contents surrounded by the intine protrude, and a small papilla which is colourless at the tip is soon formed. Figure 4 shows the proximal pole of a germinating spore. The first wall cuts off the small papilla in such a way that it has a broad base and contains several chloro-



Figs. 1–3.—Freshly shed spores of *B. nudum*. Fig. 1.—Proximal view showing laesura. ×460. Fig. 2.—Distal pole, chloroplasts, and oil. ×460. Fig. 3.—End view. ×345. Figs. 4–20, 22–24.—Stages in germination and development of the prothallus on mineral solution. Fig. 4.—Germinating spore, 4 days. ×300. Figs.

[*For continuation see opposite page*]

plasts (Fig. 5), and this elongates to form the first rhizoid (Fig. 6). A second rhizoid may subsequently develop from the basal cell (Figs. 8, 13, 17).

The next division in the primary cell is approximately perpendicular to the first, and cuts off the first cell of the filament (Fig. 7). Occasionally the first two divisions are parallel, but as they are perpendicular to the long axis of the spore, germination cannot be described as bipolar (Figs. 10, 11).

Figures 8–20 and 22–25 show various stages in the development of the young prothallus.

The filament varies somewhat according to the external conditions and a filament of two to three short cells is usually formed (Figs. 9, 10), but five very narrow cells were occasionally produced in cultures subjected to weak light. When light conditions were favourable, a longitudinal division frequently occurred in the first cell of the filament, next to the basal cell, and plate formation was initiated immediately (Figs. 8, 20).

The wedge-shaped apical cell usually develops in the terminal cell of the filament. An obliquely longitudinal wall is produced and the larger of the resultant cells is then divided by a wall which cuts the first septum approximately at right angles (Figs. 9, 10). Longitudinal walls may subsequently form in cells farther back.

The early sequence of divisions is not always the same and sometimes the terminal cell is first divided by two walls which do not intersect (Fig. 14) and occasionally the first longitudinal division occurs in the penultimate or a cell farther back from the apex (Fig. 11).

Lateral segments cut off from the apical cell grow rapidly and divide so that the outer cells are pushed forward and the apical cell comes to occupy a slight depression (Fig. 23–25).

Only occasionally does the apex become very indented as in Figure 26. In this prothallus the apical cell has divided by a periclinal wall followed by an anticlinal wall, initiating the formation of a row of marginal initials which are shown at a later stage of development in the apex in Figure 79.

Figure 35 shows nuclear divisions at the apex of a small prothallus with a slight indentation. Lateral segments of the apical cell are active and periclinal and anticlinal divisions are taking place.

A rather characteristic feature of the prothallus is the formation of an oblique wall in one of the lower cells of the filament. Figure 9 shows a protuberance on the

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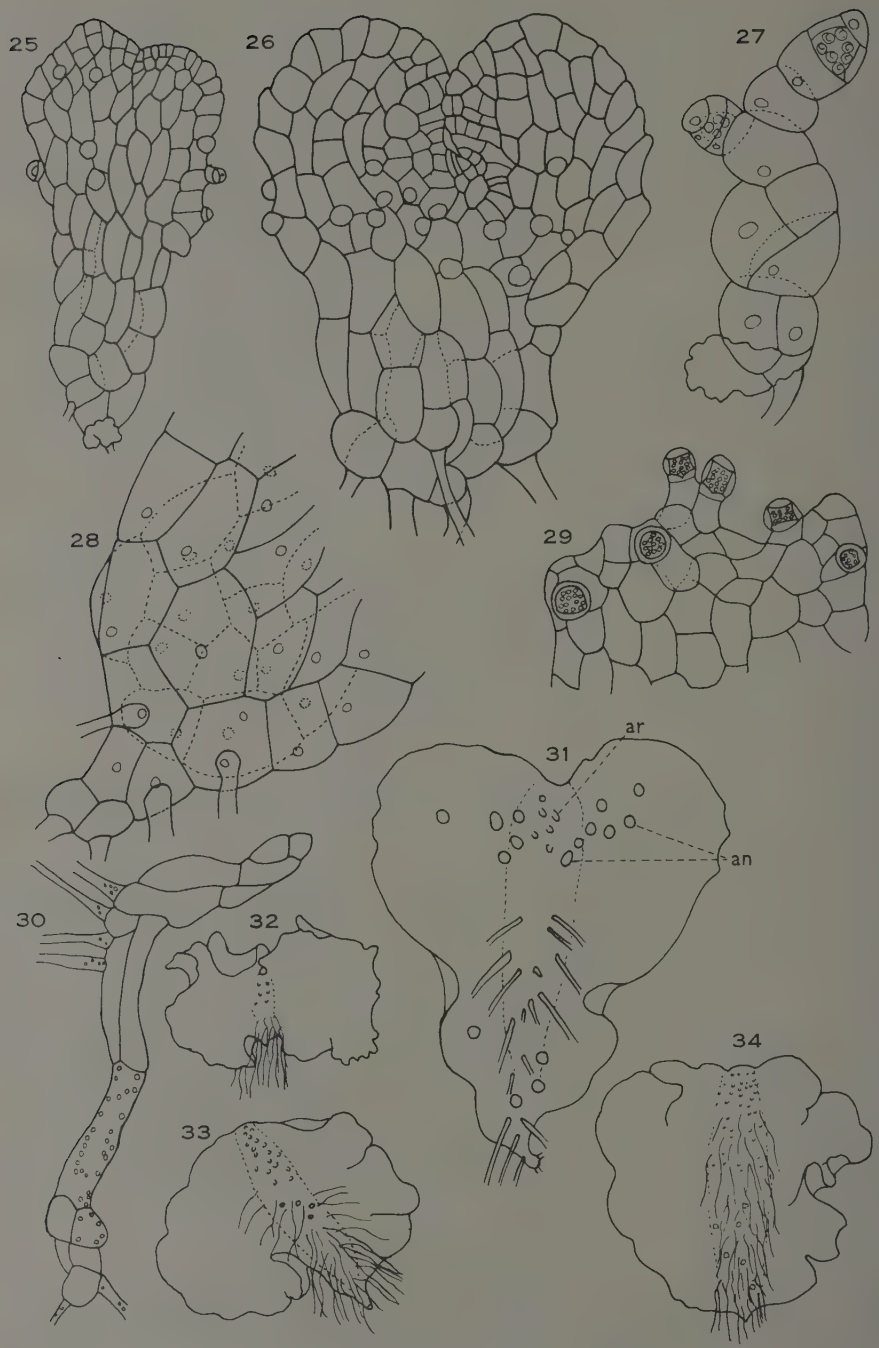
Figs. 1–24 (*Continued*)

5–8.—9 days. ×300. Fig. 5.—Cleared, rhizoid delimited, perispore shed, exine persistent. Fig. 8.—Cleared. First longitudinal division. Second rhizoid cut off from basal cell. Figs. 9–16, 20.—Development of apical cell and early stages of plate formation, 14 days. ×160. Figs. 17–19.—21 days. ×150. Fig. 19.—Cleared. Divisions in three planes. Fig. 21.—Rhizoid tip from gametophyte on boiled *Sphagnum*. ×150. Fig. 22.—21 days. ×120. Figs. 23, 24.—2 months on mineral solution and perlite. Development of apical sinus. ×110.



Figs. 25–34.—Gametophyte of *B. nudum*. Fig. 25.—Ventral surface of small prothallus with thick base, marginal and superficial antheridia, 2 months. × *c.* 50. Fig.

[*For continuation see opposite page*]

cell next to the basal cell in preparation for this and Figures 13, 18, and 22 show the oblique wall formed. The cell produced contributes to the broad base of older prothalli. An oblique wall may also form in a marginal cell near the apex of a prothallus producing a wedge-shaped cell as in Figure 18. A similar cell can be seen in older prothalli shown in Figures 23 and 24.

There is a strong tendency for the basal part of the prothallus to become massive. Divisions in a third plane often occurred quite early in development and this was particularly noticeable in cases where no definite apical cell could be determined (Figs. 16, 19). Even small prothalli such as those in Figures 24–26 usually had a thick base, only the basal cell remaining small and as a rule undivided. Figure 28 shows the detail of the proximal end of a small prothallus; the distal portion was one cell thick.

The basal cell characteristically remains small, short, and bulbous. If the cell next to the basal cell remains undivided, it is usually very robust and much larger than the latter (Figs. 13, 14). The cell next to the basal cell, however, divides in almost all cases, even in ameristic prothalli produced in very crowded conditions (Figure 27 shows one in which even the basal cell is divided), and in elongated prothalli grown in distilled water for 3 months (Fig. 30).

Individual marginal cells project so that the margin of the prothallus is not smooth (Fig. 26) and older prothalli have undulate edges and irregular bulges often in the apical region as seen in Figures 31, 34, and 79. No hairs, however, were seen at any stage of development.

The sinus is rarely deep and is sometimes absent in mature gametophytes (Fig. 33).

Rhizoids when first cut off contain a few chloroplasts (Figs. 5, 7); these do not degenerate but usually increase in number and in a young rhizoid are quite conspicuous, occurring in groups in the vacuolate cytoplasm (Fig. 6). Chloroplasts persist for some time even when the rhizoid turns brown. Rhizoids are at first marginal (Fig. 24), but when the base of the prothallus thickens they develop from cells on the ventral surface (Fig. 26).

Prothalli grown on *Sphagnum* develop rhizoids with swollen and irregularly shaped tips (Fig. 21) which are firmly attached to the moss leaves. When grown on perlite, mature prothalli have comparatively few rhizoids of a lighter colour than those produced on other media (Fig. 31).

Figs. 25–34 (*Continued*)

26.—Similar, but with a deep apical sinus. ×75. Fig. 27.—Prothallus grown under crowded conditions, seven cells, two antheridia. × *c.* 200. Fig. 28.—Thick base of small prothallus cleared. ×360. Fig. 29.—Adventitious branch from submerged prothallus, 3 months. ×80. Fig. 30.—Irregular prothallus, with scattered pale chloroplasts, on distilled water, 3 months. ×80. Fig. 31.—Mature prothallus, shallow sinus, antheridia (*an*), archegonia (*ar*), 6 months. ×20. Fig. 32.—Mature prothallus, 3 months. ×6. Fig. 33.—Mature prothallus, no apical sinus, 4 months. ×8. Fig. 34.—Mature prothallus, 12 months. ×4.

There is a thick mat of stout, rather thick-walled, reddish brown rhizoids present on the elongated cushion area of gametophytes which are one year old (Fig. 34).

Prothalli soon develop antheridia (Figs. 25, 26) on the margin, the ventral, and sometimes the dorsal surface, then archegonia after 2–6 months (Figs. 31–33).

The time taken to reach maturity and the shape of the prothallus vary with the month of sowing. Archegonia generally develop in the summer months and prothalli grown from spores sown in October produce archegonia in 2–3 months (Fig. 32) whereas prothalli from the August plantings take 4–6 months and are more elongated (Fig. 31). Archegonia often form on both surfaces in the cushion area when prothalli adopt a vertical position on the substrate. Prothalli on distilled water did not reach maturity and when grown on *Sphagnum* were slower in producing sex organs than those on other media. Figures 80 and 81, which are sagittal sections of young mature prothalli 4 months old, show that the cushion is 3–5 cells thick when archegonia develop. Archegonia form 5 or 6 cells from the apex, and in the section shown in Figure 81 there is an antheridium in close proximity to an archegonium.

Gametophytes which are one year old are more elongated and the wings more ruffled (Fig. 34). They develop thicker cushions of 7–10 cells, shown in sagittal section in Figure 82 and transverse section in Figure 77. Figure 93 is portion of a transverse section of a prothallus bearing an archegonium at the margin of the cushion and showing the junction of the cushion and the wing, which is 3 or 4 cells thick in this region.

Sections of the cushion region reveal that 7 or 8 cells back from the apex the cushion reaches its maximum number of cells in depth. Further back the cushion is much deeper but only because the cells are larger.

Gametophytes with thin cushions develop fewer archegonia than old heavy ones, on which archegonia are very numerous. Antheridia frequently develop with archegonia on the cushion (Figs. 79, 81) and these may be extremely large (Fig. 72), but a few prothalli have no antheridia (Fig. 32) or only a few in the proximal region.

Irregularities may occur as in Figure 78, which shows a one-year-old prothallus in which the midrib has extended as a cylindrical process bearing many antheridia and some archegonia and rhizoids.

Crowded cultures on perlite, *Sphagnum*, and unsterilized *Dicksonia* roots show a great development of small irregular and cordate prothalli which produce a tremendous number of antheridia. Some slender ameristic prothalli with as few as seven cells bear antheridia (Fig. 27), and there are often two or three antheridia to a cell.

Prothalli which are submerged in mineral solution produce numerous adventitious branches which grow above the surface of the solution and later develop antheridia abundantly (Fig. 29).

IV. Reproductive Organs

(*a*) *Antheridia*

The abundance of antheridia on the margins and surfaces of small ameristic prothalli gave ample material of a suitable nature for a detailed examination of antheridial development, and Figures 36–76 are a representative selection chosen from the 136 which were drawn.

The antheridium arises from a prothallial cell as a protuberance, which forms above the nucleus which is enlarged and surrounded by denser cytoplasmic material than the rest of the cell (Figs. 36, 37).

Mitosis occurs and a wall forms in a plane parallel with the surface of the prothallus if it is a superficial antheridium (Fig. 39), or perpendicular to the surface if it is a marginal antheridium (Fig. 40). In telophase the two newly formed nuclei usually appear unequal in size, the smaller one remaining in the mother prothallial cell, the larger, with dense cytoplasm and some chloroplasts, in the small protuberance which is cut off by the new dividing wall (Fig. 38).

A comparison of Figures 35 and 38 shows equal nuclei at telophase in an ordinary somatic division (Fig. 35) and inequality in those where an antheridium is forming (Fig. 38). The nucleus in the antheridial initial continues to enlarge and may be twice the size of the somatic nucleus (Fig. 39).

Mitosis occurs in the nucleus of the antheridial initial and at telophase the denser cytoplasm is in the outer part of the antheridium, the inner part being vacuolate (Fig. 41). The mitotic axis is in the same plane as that of the previous division (Figs. 40, 41) and a wall is laid down parallel with the first wall and the inner, and usually smaller, of the two new cells becomes the basal cell of the antheridium. The denser cytoplasm is more concentrated in the outer cell and its nucleus becomes larger (Fig. 42).

The nucleus of the outer cell divides (Figs. 43–46) and at telophase the denser cytoplasm is more concentrated in the lower central part of the cell, the outer periphery being clearer (Figs. 45, 46). The wall which is laid down is domed and surrounds the denser cytoplasm and the inner nucleus (Figs. 47, 48). As the mitotic axis is in most cases not quite perpendicular to the wall separating the basal from the outer cell (Fig. 43 and Plate 2, Fig. 2), the nucleus of the new outer or wall cell is not centrally placed at the top of the cell, but is in a slightly lateral position (Figs. 47, 48, and 51).

The nucleus of the inner or androgonial cell becomes much larger than that of the wall cell which surrounds its outer periphery (Figs. 47, 48) and sometimes the androgonial cell, which is generally hemispherical, presses slightly into the basal cell and so appears lens-shaped.

The nucleus of the wall cell undergoes a division which is approximately at right angles to that of the last division described and produces the nuclei of the cap and

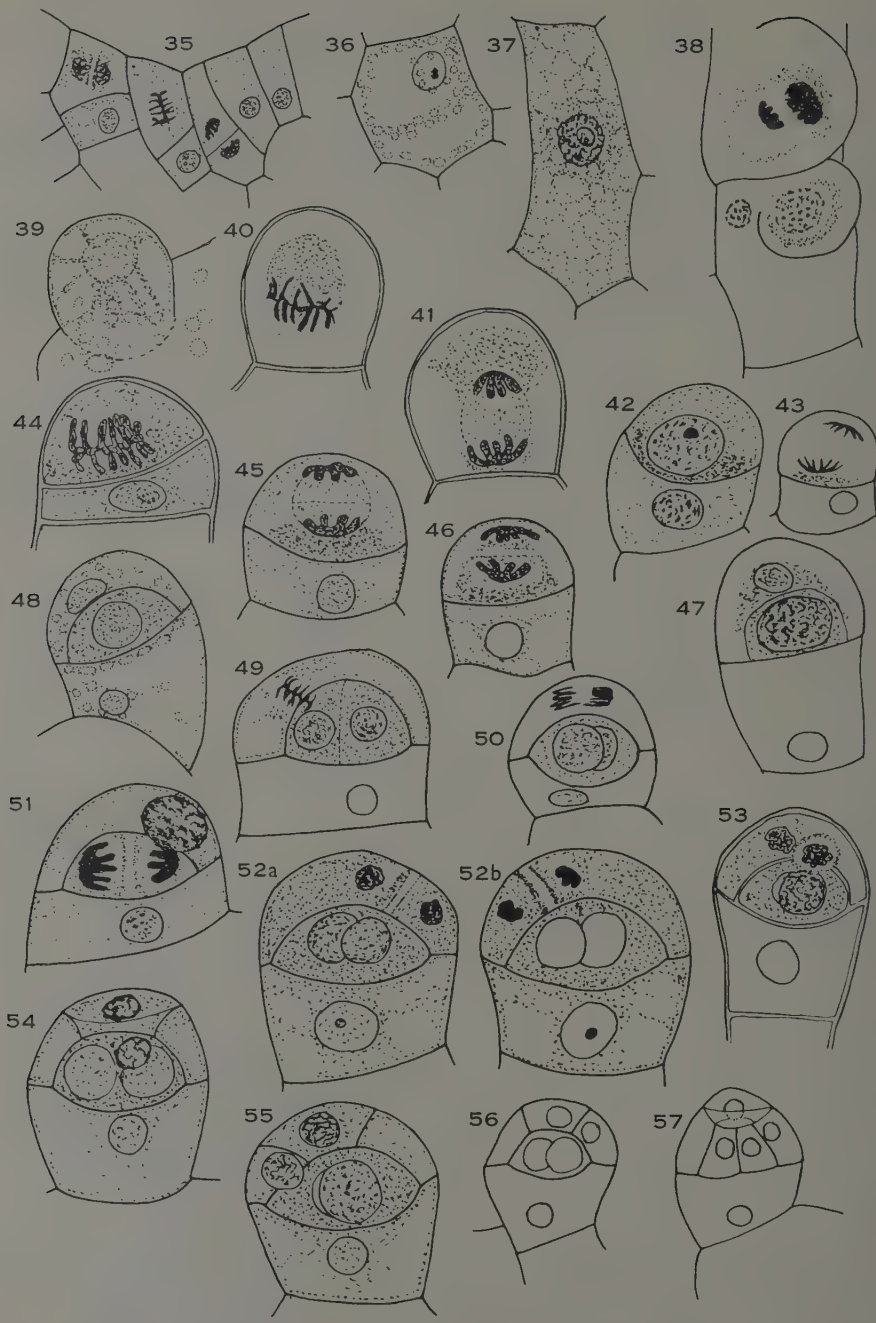


Fig. 35.—Nuclear divisions in the apical region of a small prothallus, surface view. ×460. Figs. 36–57.—Stages in antheridial development, optical sections. Figs. 36, 37, 39, and 48 fixed with F.P.A., the rest cleared with chloral hydrate. Figs. 36, 37.— Mother cells of antheridia. Fig. 36 ×300. Fig. 37 ×460. Fig. 38.—Division of nucleus of

[*For continuation see opposite page*]

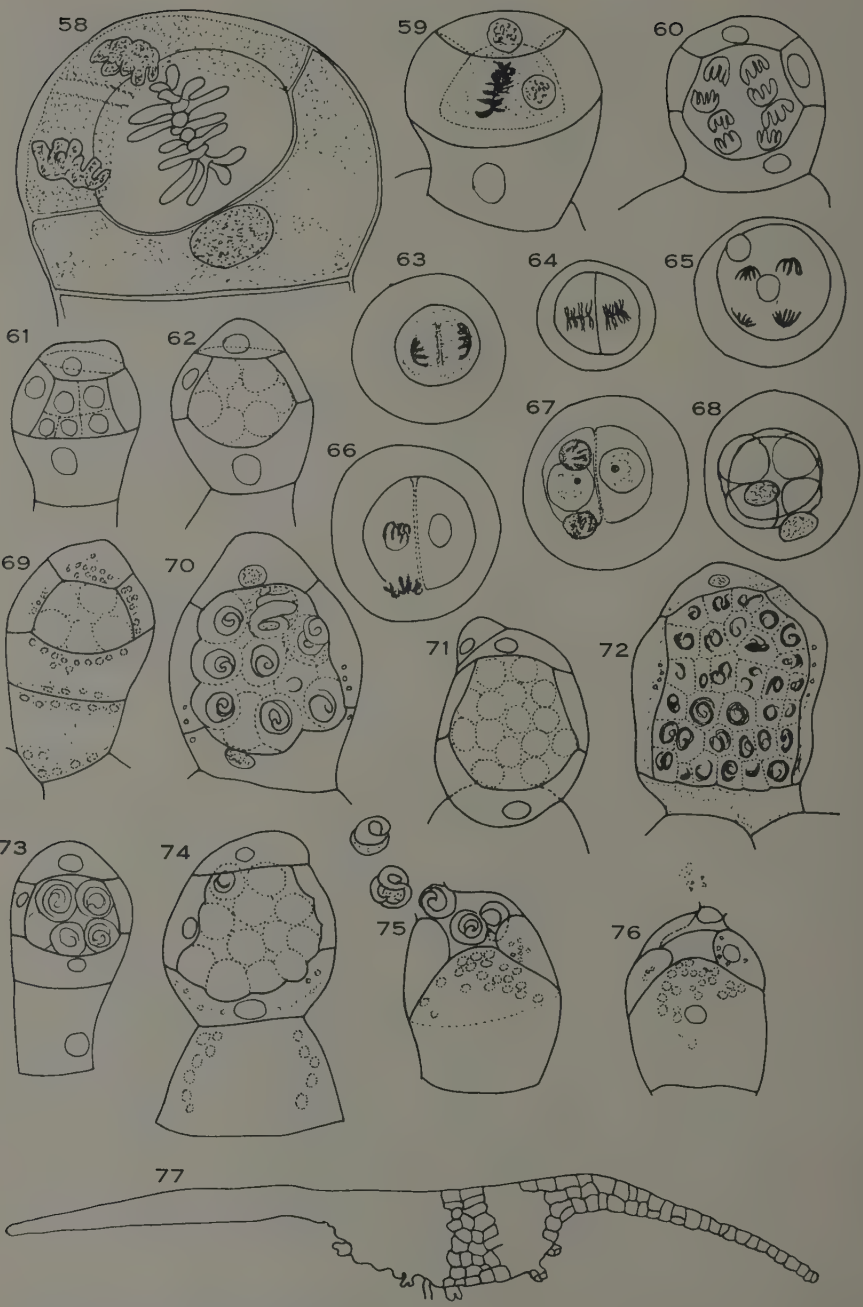
ring cell (Figs. 49, 50, 52*a*, 52*b*, 53, 58, and Plate 1, Fig. 1). The formation of the wall which delimits the cap and ring cell is not easy to follow as the wall does not stain at all readily and the nuclei, being large, obscure it unless the antheridium is lying in a particularly favourable position. Several antheridia in which the wall had partially formed were observed, in some cases from both sides. The phragmoplast is formed between the two daughter nuclei at telophase and a cell plate forms along its equatorial plane, extending from the outer wall of the antheridium to the wall enveloping the androgonial tissue. In Figure 53 the phragmoplast can be seen as a halo surrounding the cell plate forming between the two daughter nuclei. Figures 52*a* and 52*b*, which are drawings of the same antheridium viewed from both sides, show the wall nucleus at telophase and the phragmoplast and partial delimitation of the two protoplasts. Plate 1, Figure 1, shows an antheridium at a similar stage and Plate 1, Figure 2, the same antheridium at a higher level of focus, in which the wall nuclei are out of focus and the new cell plate is not apparent, nor is any delimitation of the two protoplasts. Plate 2, Figure 1, shows the same antheridium photographed from the reverse side and the level of focus as in Plate 1, Figure 1. Figure 58 is the same view of the antheridium observed with an oil immersion lens.

By the time the two daughter nuclei are reconstituted the upper nucleus is encircled by the new cell wall, which extends from the outer antheridial wall to the wall surrounding the androgonial tissue (the funnel-shaped wall of earlier workers on this subject). Figure 54 is a three-dimensional drawing showing two very young daughter nuclei and the cell plate between cap and ring cell completed. Whether a membrane was produced between the cap cell nucleus and the androgonial tissue could not be determined but at no time was the cell plate seen as a transverse wall with a gap between it and the dome-shaped wall, as described and figured by Davie (1951) for *Pityrogramma calomelanos* (L.) Link and other ferns.

The androgonial tissue is lens-shaped or hemispherical with a domed upper surface when the cap cell is formed. Figure 55 is an optical section and Figure 54 a three-dimensional view showing the newly-formed wall delimiting the cap and ring cell, and the domed upper surface of the androgonial tissue is still apparent. Figures 56, 57, and 61 are successively older antheridia and illustrate a flattening of the domed upper surface of the androgonial tissue, the alteration in shape resulting from growth and the restriction imposed by the junction between the domed and the funnel-shaped wall. At this stage the androgonial tissue frequently appears like a trapezium in optical section (Figs. 57, 61).

Figs. 35–57 (*Continued*)

mother cell to form antheridial initial cell. ×460. Fig. 39.—Antheridial initial cell. ×575. Figs. 40–42.—Division of nucleus of antheridial initial cell to form basal and outer cells. ×690. Figs. 43–48.—Divisions of nucleus of outer cell to form central androgonial cell and enveloping wall cell. Fig. 43 ×460; Figs. 44, 45, 47 ×690; Figs. 46, 48 ×575. Figs. 49–57.—Division of nucleus of enveloping cell to form ring and cap cell. Figs. 52*a* and 52*b* are views of the same antheridium from opposite sides. Figs. 49, 51, 52, 54, 55 ×690; Figs. 50, 53 ×575; Figs. 56, 57 ×460.



Figs. 58–76.—Antheridia. Fig. 58.—Division of nucleus of enveloping cell and of androgonial cell, oil immersion. × *c.* 900. The same antheridium as shown in Plate 1 and Plate 2, Figure 1. Figs. 59–62, 69, 70.—Stages in development of androgonial tissue. Figs. 63–68.—Surface views of developing antheridia showing nuclear divisions in

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The first division of the androgonial cell usually occurs just before (Figs. 49, 51), sometimes simultaneously with (Fig. 58), and rarely after (Fig. 59) the division of the wall nucleus to form cap and ring cells. The relationship between the division of the wall and androgonial nuclei is shown by a comparison of Figures 66 and 67, which are surface views, and Figures 50, 54, and 55, which are side views. The axes of the mitoses are approximately at right angles to each other but not in the same plane.

The first division in the androgonial cell is in a plane parallel with the longitudinal axis of the antheridium (Figs. 49, 51, 63, 66, 67), and the division between the two daughter nuclei is also parallel with the axis of the antheridium but approximately at right angles to the first division (Figs. 64, 65, 68). The next divisions may be perpendicular to the axis of the antheridium (Figs. 60, 61), but subsequent divisions are not always regular and the number of sperms varies in longitudinal section from 4 to 5 in very small antheridia to 8–12–16 or over 30 in some large antheridia found on cushions (Figs. 70–74).

At maturity the cap cell is asymmetrical, having a bulge or beak on one side in almost all cases, although this asymmetry is not apparent from all angles (Figs. 69, 70). If a culture is viewed with a dissecting microscope it is seen to be particularly characteristic and not just a feature which arises during dehiscence as Stokey and Atkinson (1952*a*) reported for *B. buchtienii*. The feature may appear before the antheridium is mature as seen in Figure 61. Only occasionally is the cap cell divided and Figure 71 shows an anomalous division in the cap cell cutting off the bulge. It would be unlikely that such an antheridium would dehisce.

Large antheridia on the cushion sometimes had two ring cells, but this feature was not observed in any of the antheridia on small prothalli.

The basal cell may be short (Fig. 72) or elongated, and though often depressed by the sperm cells (Figs. 62, 74), is never funnel-shaped. It may have a broad base but is usually slightly constricted in this region (Figs. 70, 74). One or more antheridia may be borne on a cell projecting from the surface (Fig. 74) or margin (Fig. 73) of the prothallus.

The mature antheridium usually has an elongated appearance and chloroplasts, present in the wall cells, are smaller than in the somatic cells and inconspicuous, as they are mostly arranged on the surfaces of the walls which surround the androgonial tissue.

Dehiscence of the antheridium was observed and was essentially similar to that described by Stokey and Atkinson (1952*a*) for *B. buchtienii*. There is an upward

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Figs. 58–77 (*Continued*)

androgonial tissue. Figs. 65–68.—Division of nucleus of the enveloping wall cell to form ring and cap cell. Figs. 70–74.—Mature antheridia showing variation in the number of sperm cells. Fig. 71.—Anomalous division of the asymmetrical cap cell. Fig. 72.—Longitudinal section of large antheridium. Figs. 75, 76.—Dehiscence. Fig. 77.—Cross section of gametophyte, 1 year. × *c.* 50. Figs. 59, 63, 66–68 ×575; Figs. 60, 70 ×690; Figs. 61, 62, 65, 69, 71–76 ×460; Fig. 64 ×375.

Figs. 78–93.—Archegonia of *B. nudum*. Fig. 78.—Prothallus with elongated cylindrical process bearing antheridia and archegonia, 15 months. ×3. Fig. 79.—Ventral surface of apical region of prothallus, cleared. Marginal meristem, archegonia, and

[*For continuation see opposite page*]

pressure of the basal cell and inward pressure of the ring cell and the spermatocytes which previously only indented the cap cell suddenly enter it, the cuticle ruptures at one side of the cell in the region of the bulge, and the sperm cells quickly emerge one at a time through a pore (Fig. 75). A few plastids and the nucleus of the ruptured cap cell are also extruded (Fig. 76). After a number of cells have been set free there is a lull and then another series emerges; the last sperm may be extended as it escapes, and its vesicle left inside the antheridium.

Figures 75 and 76 show the position of the greatly expanded basal cell which presses up through and distorts the ring cell which also helps to force the spermatocytes out. With the change in shape of the basal cell the chloroplasts become a conspicuous feature.

(*b*) *Archegonia*

The archegonia develop in the usual manner and stages are shown in Figures 83–88 and 90. The initial cell of the archegonium (Fig. 83) can be recognized four or five cells back from the apex of the prothallus and the first two divisions (Figs. 84, 85) produce a row of three superposed cells. The outer cell divides (Figs. 86, 87) and gives rise to four rows of neck cells, and the basal cell frequently divides at an early stage (Fig. 87).

The axial row at maturity consists of two neck canal cells, a ventral canal cell, and an ovum (Fig. 90), the wall separating the ventral canal cell from the neck canal cell often being thin and rather indistinct. Occasionally archegonia with four neck canal nuclei were found on prothalli grown for four months (Fig. 89).

The neck is short, of four or five tiers, and the individual cells are also short so that the archegonium has a rather squat appearance. The neck cells have conspicuous nuclei and at maturity longitudinal and oblique divisions frequently occur in the lowest cells (Figs. 89–92), which have granular contents and stain very deeply. The neck is usually straight but may be very recurved (Figs. 81, 82) and the archegonia are alike on young and old prothalli.

The distal part of the neck canal swells somewhat in water, mucilage is present in the canal, and the neck cells at the tip become thin (Fig. 91). Figure 92 is a section of an archegonium showing sperms entering the open neck. Sporophytes were occasionally found in the cultures.

Figs. 78–93 (*Continued*)

antheridia, 1 year. ×80. Fig. 80.—Longitudinal section of apical region of prothallus with thin cushion and young archegonium, 4 months. ×220. Fig. 81.—Similar, with a thicker cushion, archegonia, and an antheridium. ×100. Fig. 82.—Longitudinal section of apex of prothallus with thick cushion, 12 months. ×220. Figs. 83–88, 90.—Longitudinal sections showing stages in the development of an archegonium. Figs. 83–85 ×300; Figs. 86–88 ×460. Fig. 89.—Longitudinal section of an archegonium with four neck canal nuclei. ×345. Figs. 90, 91.—Longitudinal sections of mature archegonia. ×460. Fig. 92.—Longitudinal section of an open archegonium with sperms entering neck canal. ×460. Fig. 93.—Transverse section of prothallus with archegonium at junction of cushion and wing, 1 year. × *c.* 150.

V. Conclusion

Blechnum nudum has a green spore which is not found in the other members of the Victorian Blechnaceae, nor has the author seen it reported for any other *Blechnum* sp. It is not a common condition in a bilateral spore, and whether it is of any significance is not known. The families with green spores, however, are usually among the more primitive, e.g. Osmundaceae and Hymenophyllaceae, and the less primitive Grammitidaceae.

B. nudum differs from the six other *Blechnum* spp. described in the literature in having a gametophyte with no hairs. Naked prothalli, however, are produced by several of the Australian *Blechnum* spp. which the author has in culture at present, namely: *B. procerum* (Forst.) Sw., *B. minus* (R.Br.) Ettings, *B. patersoni* (R.Br.) Mett., and *B. penna-marina* (Poir.) Kuhn, while *B. fluviatile* (R.Br.) Lowe ex Salomon and *B. cartilagineum* Sw. bear papilla-like hairs and similar hairs are also present in *Doodia aspera* R.Br. Stokey (1951) reports that in the genera *Athyrium* Roth and *Asplenium* L. hairs are present on the gametophytes of some members and not on others.

In *B. nudum* the filament is never terminated by a papillate hair with a lateral development of the apical cell as usually occurs in species described and reviewed by Stokey and Atkinson (1952*a*) but the apical cell develops from the terminal cell as in *B. orientale* (Kachroo 1955) and *B. brasiliense* (Jung 1927, not seen).

In *B. nudum* there is a strong tendency towards early plate development and only very rarely is a long filament formed. In this it resembles the Cyatheaceae and other primitive families.

Divisions in a third dimension producing a thick base may occur in quite small prothalli. This is a feature characteristic of the Osmundaceae (Stokey and Atkinson 1956), but in *B. nudum* the basal cell is rarely divided and germination never bipolar as in that family. The condition could be a primitive one, or possibly only associated with its terrestrial habit, but it has not been described for any other of the *Blechnum* spp.

Rhizoids which contain chloroplasts are a conspicuous feature of the prothallus of *B. nudum* and this is a characteristic frequently present in primitive ferns (Stokey 1951).

The antheridium of *B. nudum* has the basal, ring, and cap cells characteristic of the advanced ferns, but it has a rather elongated appearance and shows considerable variation in the shape of the basal cell, which is usually disk-shaped, sometimes columnar, and may be asymmetrical, but is never funnel-shaped. When the antheridial initial divides, the basal cell is usually the smaller of the two cells produced whereas in *B. buchtienii* (Stokey and Atkinson 1952*a*) the lower cell is the larger.

Sometimes the antheridium is very large with a great number of sperms, but the wall structure is still of a simple type.

Stokey and Atkinson (1952*a*) report an elongated antheridium with columnar basal cell for *B. spicant* and *B. buchtienii*, whereas Kachroo (1955) reports a slightly elongated antheridium with a funnel-shaped basal cell which does not have a stalk-like appearance in *B. orientale*.

The asymmetrical cap cell of the antheridium of *B. nudum* has not been described for the other *Blechnum* spp. and Stokey and Atkinson (1952*b*) report that an asymmetrical cap cell has only been recorded for the genus *Acrostichum*.

Dehiscence of the antheridium in *B. nudum* is essentially the same as described by Stokey and Atkinson for *B. buchtienii* and not like that of *B. orientale* (Kachroo 1955) where the whole cap cell was thrown off.

B. nudum develops a cushion 7–10 cells thick on one-year-old prothalli and Stokey and Atkinson report the same feature in old prothalli of *B. spicant*. In addition, older prothalli of *B. nudum* frequently have an extension of the cushion area beyond the wings as reported for some Cyatheaceae (Stokey 1930).

B. nudum has an archegonium with a very short neck which is a characteristic of advauced ferns, but the neck is rather straight and four neck canal nuclei, common in the archegonia of the Cyatheaceae and some other ferns, are occasionally present.

The prothallus of *B. nudum* therefore has several vegetative characters which are usually associated with the primitive ferns, but in its sexual organs exhibits features of the advanced ferns. Vegetatively it has little resemblance to the *Blechnum* spp. previously described in the literature but it does resemble those described by Stokey and Atkinson in the elongated form and dehiscence of the antheridium.

VI. Acknowledgments

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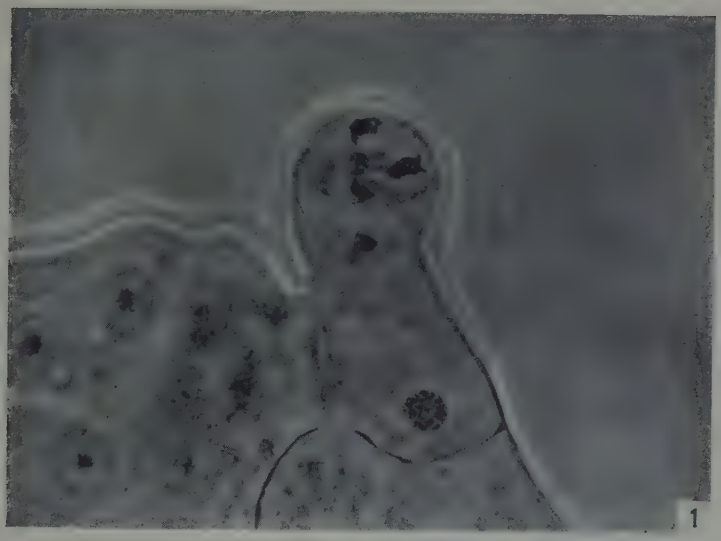
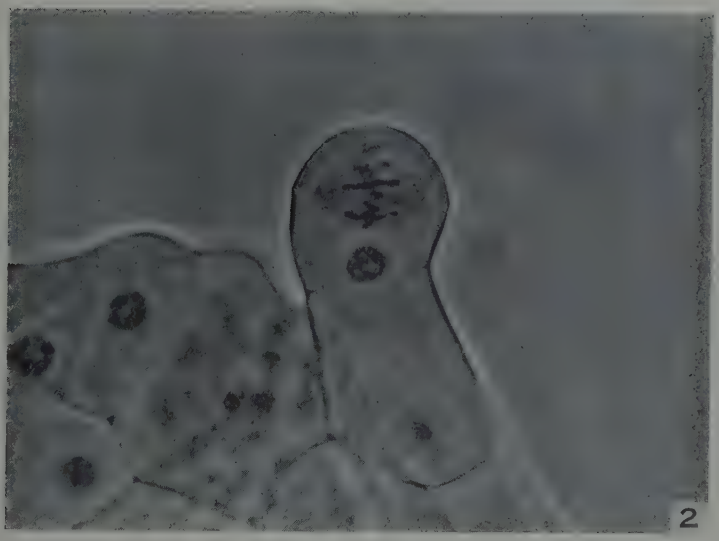
GAMETOPHYTES OF THE VICTORIAN BLECHNACEAE. I



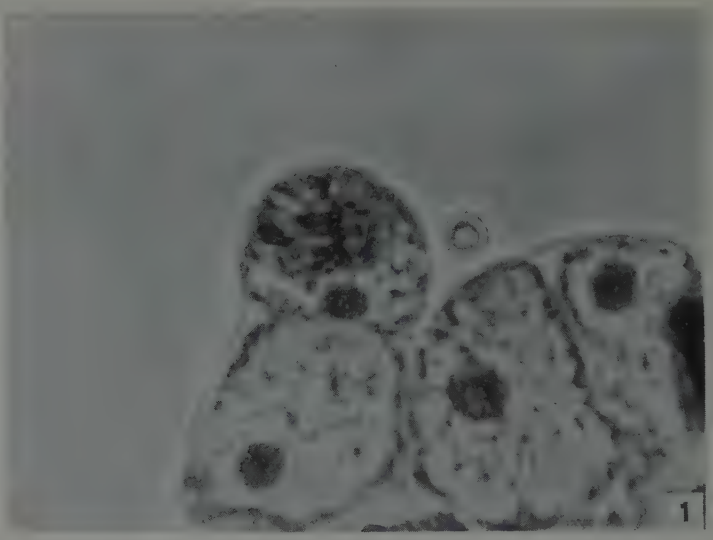


Optical sections of an antheridium of *B. nudum* in which the androgonial nucleus is at metaphase, and the nucleus of the outer cell is at telophase. Phase contrast. × *c.* 600.

Fig. 1.—Note newly-formed nuclei of the cap and ring cell with phragmoplast and beginning of cell plate between them.

Fig. 2.—Higher focus; metaphase of androgonial nucleus sharply defined, wall nuclei out of focus and cell plate not showing at this level.

GAMETOPHYTES OF THE VICTORIAN BLECHNACEAE. I



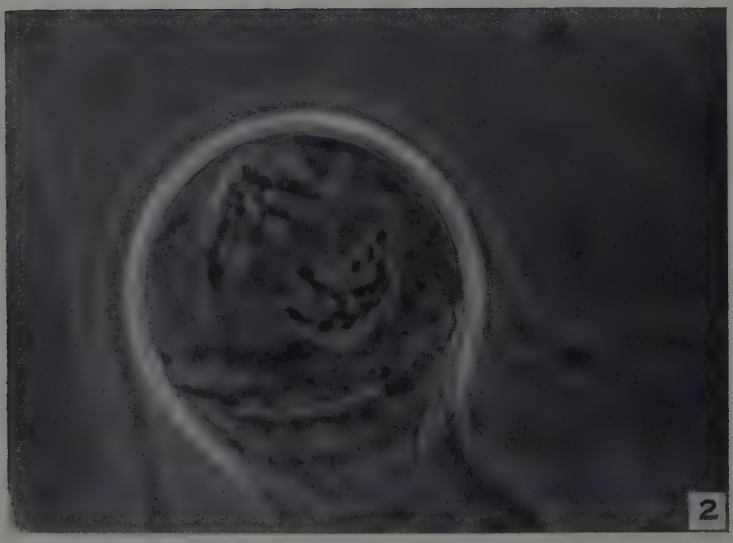


Fig. 1.—The same antheridium as shown in Plate 1, photographed from the opposite side. The level of focus is the same as in Plate 1, Figure 1. × *c.* 600.

Fig. 2.—Young antheridium showing late anaphase in the outer cell. The axis of the mitosis is not perpendicular to the wall separating the outer cell from the basal cell. × *c.* 1200.

THE EFFECTS OF TEMPERATURE AND DAY LENGTH ON SPECIES OF *EUCALYPTUS*

By G. Scurfield*

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Summary

Seedlings of *Eucalyptus bicostata, E. blakelyi, E. niphophila, E. pauciflora, E. polyanthemos, E. rubida,* and *E. stellulata* were grown under various defined temperature and day-length regimes and response was assessed in terms of height, dry weights of stems, roots, leaves, and branches, number of leaves and branches, and leaf shape.

Seedlings of *E. bicostata, E. blakelyi, E. niphophila, E. pauciflora,* and *E. rubida* were capable of continuous growth under short days (8 hr) provided temperature conditions were appropriate. These species can be allocated, therefore, to Class C of Nitsch (1957). Growth was greater under long days of 18 hr than under short days of 8 hr.

Irrespective of the temperature regime operating, the order of dry weight increase over 60 days was: *E. bicostata*>*E. blakelyi*>*E. polyanthemos*>*E. niphophila*. Growth of *E. bicostata, E. blakelyi,* and *E. polyanthemos* increased as mean temperature levels increased thus: 14/8°C, 19/11°C, 20/17°C, 25/20°C. Further increase to 27/24°C had an adverse effect upon growth of *E. polyanthemos*, but not on that of *E. bicostata* and *E. blakelyi*.

Increasing night temperature from 6°C to 24°C when the day temperature was either 11, 20, 24, or 28°C generally increased height, dry weights of stems, roots, and leaves, and total leaf area of *E. blakelyi*. Similar effects were produced when day temperatures were increased from 11°C to 28°C, night temperatures being either 6, 16, 19, or 24°C. Consequently, under short days of winter, the 28/24°C regime was most nearly optimal for growth. Adverse effects on growth appeared to be produced when night temperature exceeded a low day temperature (as under the 11/24°C regime). Average day and night temperature interacted in a statistically significant way upon growth.

In *E. pauciflora,* increasing night temperature from 8°C to 24°C when the average day temperature was either 14, 20, 25, or 27°C, and increasing day temperature from 14°C to 27°C when night temperature was 8, 17, 20, or 24°C increased growth. Thus, the regime 27/24°C approached the optimum for growth, as in *E. blakelyi*.

In *E. blakelyi* and *E. pauciflora,* branching was most restricted in plants which were tallest. There were indications that the magnitude of diurnal temperature fluctuations might be important in determining the degree of lateral branching (apical dominance).

Leaf size, shape, and number were related to day length and temperature conditions. Leaf shape (as measured by length/breadth ratios) was a function of day and night temperatures, not being significantly related to either alone. Day length appeared to exert a more pronounced effect upon leaf shape (long days tending to shift shape in the direction juvenile→adult) than temperature.

The ratios top/root and leaf/stem, especially the latter, were significantly lower in *E. blakelyi* than in *E. pauciflora*.

* Division of Plant Industry, C.S.I.R.O., Canberra; now at Division of Forest Products, C.S.I.R.O., South Melbourne.

I. Introduction

The present investigation is concerned with the influence of environmental factors on the early growth of species of *Eucalyptus*. Experiments reported here deal with the effects of temperature and day length. The aim is to provide a physiological basis for assessing the performance, distribution, and morphological characteristics of such species. Apart from the data given by Kramer (1957, 1958) for *Pinus taeda* L. and by Hellmers (1959) for *Pinus lambertiana* Dougl., there appears to be little quantitative information on the effects of temperature on the growth and morphology of woody perennials. Regarding the effects of day length, Nitsch (1957) in a recent review classified 102 woody species according to their growth response to photoperiod. None of the species listed was native to Australia. From a comparative point of view, therefore, the growth response to photoperiod of species of *Eucalyptus* was of interest.

II. Methods

Seedlings of the following species of *Eucalyptus* were used in various experiments: *E. bicostata*, *E. blakelyi*, *E. rubida* (Section Macrantherae), *E. niphophila*, *E. pauciflora*, *E. stellulata* (Section Renantherae), and *E. polyanthemos* (Section Terminales). They were chosen as representatives of different taxonomic groups with the further consideration that they differed in their altitudinal range. Listed in the order of their upper altitudinal limits, the sequence of species would probably be *E. niphophila*, *E. pauciflora*, *E. stellulata*, *E. rubida*, *E. bicostata*, *E. polyanthemos*, and *E. blakelyi*, with *E. niphophila* having the highest limit (about 6500 ft).

(*a*) *Temperature Experiments*

The following environments, differing in their prevailing temperatures, were available: outside (Canberra winter) conditions, an unheated glasshouse without temperature control, and three air-conditioned glasshouses. The last were constructed with the intention of maintaining temperature regimes of 20/15°C,* 25/20°C, and 28/25°C respectively. The actual average day and night temperatures attained during the various experiments are shown in the tables of results given below.

(i) *Experiment* 1.—Twelve seedlings (at the cotyledon stage of growth) each of *E. bicostata*, *E. blakelyi*, and *E. polyanthemos*, growing singly in pots, were placed in each of the five environments. The number of seedlings of *E. niphophila* available at the time were sufficient only for placing in two environments—the unheated glasshouse and the air-conditioned glasshouse where highest temperatures were maintained. Thermographs were used to obtain a continuous record of air temperature on all sites. Rates of height growth were compared over a period of 60 days and performance assessed at the end of that time.

(ii) *Experiment* 2.—In order to assess the relative importance of average day and night temperatures in the growth of species of *Eucalyptus*, plants of *E. stellulata*, *E. blakelyi*, and *E. pauciflora* were grown under 16 combinations of day and night temperatures.

* Throughout the text, the "denominator" refers to average night and the "numerator" to average day temperature in °C.

Day temperatures were maintained between 8.30 a.m. and 4.30 p.m., and night temperatures for the remainder of each day. Certain regimes were obtained by moving plants (at 8.30 a.m. and 4.30 p.m.) appropriately between outside conditions and the three air-conditioned glasshouses. It should be borne in mind, when comparing the growth of plants under outside and glasshouse conditions, that any regime attained by placing plants outside during day or night, or both, involves confounding the possible effects of other environmental factors upon growth with those of temperature. There were 12 replicates per treatment, the plants growing singly in pots.

(*b*) *Day-length Experiments*

Experiments were conducted with seedlings (growing singly in pots) of *E. blakelyi*, *E. niphophila*, *E. pauciflora*, and *E. rubida*, and with plants (approximately 38 cm high initially) of *E. bicostata*. Equal numbers of plants of each species were placed under long days (*LD*) of 18 hr (achieved by lengthening normally short winter days with light from incandescent lamps providing 25–30 f.c. of illumination at bench height) and short days (*SD*) of 8 hr (8.30 a.m. to 4.30 p.m.) (achieved by covering the plants with light-proof curtains for 16 hr each day). These treatments were applied in a heated glasshouse in which average temperatures were maintained roughly at 16–19°C from 4.30 p.m. to 8.30 a.m. and 20–25°C for the remainder of each day.

(*c*) *Day Length–Temperature Experiments*

Factorial experiments in which plants are subjected to various combinations of day length and temperature are necessary to determine the effects of temperature–day length interactions upon growth. As phytotron facilities will shortly be available here, limited experiments only were conducted along these lines.

Twenty-four seedlings, growing singly in pots, of *E. blakelyi* and *E. pauciflora* were selected at random from large populations of seedlings of each species. Twelve of the 24 plants of each species were placed under *LD* and 12 under *SD* in the glasshouse used for day-length experiments (see (*b*) above). The remainder of the populations were placed outside under winter (natural short-day and low-temperature) conditions. From these populations, 12 plants of each species were selected at random and labelled as control groups. From the remainder, 24 plants of each species were randomly selected at intervals of a few weeks and transferred to the glasshouse. There, each group of 24 plants was divided into two groups of 12, one group being placed under *SD* and the other under *LD*.

In all experiments, measurements of the height of the main stem of each plant were made at the beginning of treatment and at weekly intervals thereafter. In experiments where plants were harvested, the routine procedure was to record the lateral branch number and number of leaves on the main stem of each plant, to divide the plants into tops and roots, and to subdivide the former into main stem, main stem leaves, lateral branches, and lateral branch leaves.

From contact photographic prints of the leaves, determinations of total leaf area were made with a planimeter and quantitative assessments of leaf shape made on leaves from comparable positions on the main stem of each plant. Roots, main

stem, main stem leaves, lateral branches, and lateral branch leaves were dried separately at 90°C and their dry weights recorded. From these results, the following weights were calculated: stems (S) = main stem+branch stems, branches (B) = branch stems+branch leaves, leaves (L) = main stem leaves+branch leaves, tops (T) = $S+L$, and total plant = $S+L+R$ (where R = root weight). Values for various ratios were also calculated: T/R, L/S, $B\times100/S+L$, S or L or R/total weight, and leaf area/total weight.

III. Results

(*a*) *Temperature Experiments*

(i) *Experiment* 1.—From the results in Tables 1 and 2, it is evident that all species were able to continue growth under the naturally short (10–11 hr) days of winter, provided temperature conditions were suitable. Compare the results obtained under outside (14/8°C) and unheated glasshouse (19/11°C) conditions: under the higher day and night temperatures prevailing in the glasshouse, growth of plants of *E. bicostata*, *E. blakelyi*, and *E. polyanthemos* was increased. This increased growth was reflected in the significant ($P<0\cdot05$) increase in plant height, weight of stems, leaves, roots, and therefore of tops and total weight, in branch number and weight, leaf number, and leaf area.

Regarding growth of the species under glasshouse conditions, plants in the uncontrolled, unheated glasshouse having average day and night temperatures of 19°C and 11°C respectively, might be compared with those in the temperature-controlled glasshouse where the average day and night temperatures were 20°C and 17°C respectively. Average day temperatures in these glasshouses were similar, but there was a difference of 6°C in average night temperature, temperature changes took place more gradually, and the diurnal temperature range was greater in the uncontrolled glasshouse. Yet for any species, growth (expressed in terms of plant height or yields (dry weight) of stems, leaves, or roots) of plants was similar under the two regimes. *E. blakelyi* showed a significant reduction in leaf weight and leaf area under the 20/17°C regime, and *E. bicostata* a significant reduction in axillary branch number. There was a consistent trend in all species, however, towards the production of smaller and fewer branches under this regime.

More valid comparisons can be made between plants grown in the three temperature-controlled glasshouses, for here the curves showing temperature–time trends were essentially similar in form. The data were most complete for *E. bicostata* and *E. polyanthemos*. Regarding the latter, growth as measured by height, by dry weight of stems, leaves, or roots, by total dry weight, or by leaf area, was significantly greater under the 25/20°C regime than under the 20/17°C regime. The numbers of axillary branches and their dry weight and the number of main stem leaves were also significantly greater under the 25/20°C regime. Under the 27/24°C regime, height, total dry weight, and dry weights of roots and stems were significantly less than under the 25/20°C regime. It thus appears that for this species, the latter combination of day and night temperatures was more nearly optimal for growth. Axillary branch number and weight were not significantly different for plants growing under the 25/20°C and the 27/24°C regimes.

Plants of *E. bicostata* were significantly taller under the 27/24°C regime than under the 25/20°C regime; leaf area and yields dry weight of stems, leaves, and roots were not significantly different. The dry weight of axillary branches was less under

TABLE 1

EFFECTS OF TEMPERATURE UPON DRY WEIGHTS OF STEMS, LEAVES, AND ROOTS OF SPECIES OF EUCALYPTUS

(Figures in brackets are the means of log transformation values*)

Average Temperature Day/Night (°C)	Weight of Stems (mg/plant)	Weight of Leaves (mg/plant)	Weight of Root (mg/plant)	Total Weight (mg/plant)
		Eucalyptus bicostata		
14/ 8	13 (0·99)	133 (2·11)	66 (1·81)	212 (2·32)
19/11	189 (2·27)	1340 (3·12)	441 (2·64)	1970 (3·29)
20/17	174 (2·23)	1315 (3·11)	494 (2·69)	1982 (3·29)
25/20	400 (2·59)	2243 (3·34)	816 (2·90)	3458 (3·53)
27/24	404 (2·60)	1876 (3·26)	1020 (3·00)	3299 (3·51)
		Eucalyptus polyanthemos		
14/ 8	1 (0)	10 (0·97)	7 (0·80)	18 (1·11)
19/11	40 (1·55)	219 (2·32)	87 (1·92)	346 (2·41)
20/17	32 (1·49)	191 (2·27)	87 (1·92)	309 (2·48)
25/20	144 (2·12)	559 (2·72)	283 (2·42)	987 (2·97)
27/24	97 (1·93)	447 (2·63)	171 (2·20)	714 (2·83)
		Eucalyptus blakelyi		
14/ 8	5 (0·64)	30 (1·42)	—	—
19/11	142 (2·14)	837 (2·91)	—	—
20/17	112 (2·02)	575 (2·74)	—	—
25/20	308 (2·46)	1550 (3·18)	—	—
27/24	316 (2·49)	1377 (3·12)	—	—
		Eucalyptus niphophila		
19/11	15 —	144 —	—	—
27/24	21 —	208 —	—	—
	Least difference for significance ($P>0·05$) between means of log transformation values:			
	(0·13)	(0·12)	(0·11)	(0·11)

* Transformation values are included in order that significant differences which cannot be transformed back in terms of the original data may be discussed. The error variance is consistent as between species.

the 20/17°C than under the 25/20°C regime, but their number was about the same. Compared with plants under the latter regime, the axillary branch number of those under the 27/24°C regime was significantly less. Thus, while no decision could be made as to whether the 25/20°C or the 27/24°C was more nearly optimal for dry

TABLE 2

EFFECTS OF TEMPERATURE UPON HEIGHT, AXILLARY BRANCH NUMBER, LEAF NUMBER, AND LEAF AREA OF SPECIES OF EUCALYPTUS

(Figures in brackets are means of log transformation values)

Average Temperature Day/Night (°C)	Height (mm)	No. of Axillary Branches	Axillary Branch Weight (B)	$\frac{B \times 100}{S+L}$	No. of Leaves on Main Stem	Total Leaf Area (sq. cm.)
			Eucalyptus bicostata			
14/ 8	27 (1·42)	3·6 (0·66)	9	6·1	8·2	206 (2·30)
19/11	102 (2·00)	7·1 (0·90)	503	32·9	14·0	2135 (3·33)
20/17	101 (2·00)	4·6 (0·70)	266	17·9	13·8	2101 (3·34)
25/20	166 (2·22)	4·5 (0·69)	636	24·1	18·2	3456 (3·53)
27/24	206 (2·31)	2·2 (0·40)	258	11·3	22·3	3832 (3·57)
			Eucalyptus polyanthemos			
14/ 8	7 (0·84)	0·5 (0·12)	(0)	(0)	5·7	17 (1·17)
19/11	62 (1·78)	5·2 (0·74)	70	27·1	11·0	439 (2·61)
20/17	55 (1·73)	3·8 (0·62)	25	11·0	10·5	385 (2·58)
25/20	162 (2·20)	7·4 (0·86)	126	18·0	15·5	1058 (3·01)
27/24	135 (2·10)	6·9 (0·90)	84	15·4	16·2	971 (2·96)
			Eucalyptus blakelyi			
14/ 8	12 (1·06)	3·8 (0·68)	7	18·9	7·6	50 (1·65)
19/11	110 (2·03)	8·1 (0·95)	424	43·3	15·0	1563 (3·19)
20/17	99 (1·98)	7·5 (0·91)	243	35·5	14·7	1222 (3·07)
25/20	201 (2·29)	7·3 (0·90)	555	29·9	16·8	2340 (3·35)
27/24	244 (2·18)	4·9 (0·73)	258	15·2	20·9	2490 (3·38)
			Eucalyptus niphophila			
19/11	20 —	4·3 —	30	18·9	6·2	269 —
27/24	27 —	2·9 —	26	11·1	7·8	375 —
Least difference for significance ($P>0·05$) between means:	(0·08)	(0·17)	Not calculated			(0·12)

weight production by *E. bicostata*, the former regime was more nearly optimal for the production of axillary branches, the latter for growth in height of the main stem. Apical dominance, in other words, was accentuated under the 27/24°C regime. How far the extent and rate of diurnal temperature fluctuations, rather than the average levels of temperature, were important in this connexion was not clear.

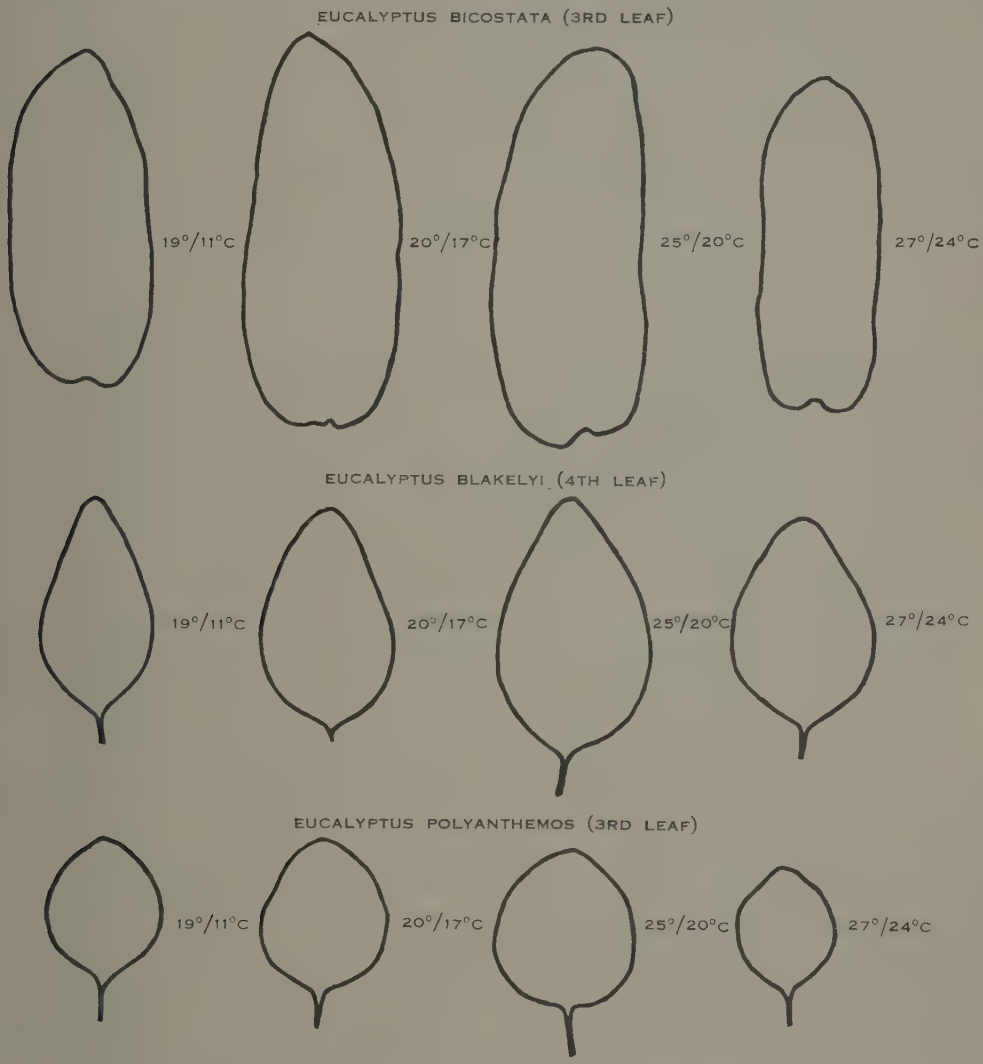

Fig. 1.—Effects of temperature upon leaf shape of species of *Eucalyptus*.

The growth responses of *E. blakelyi* to various temperature regimes were similar to those of *E. bicostata*.

Plants of *E. niphophila*, placed under the 19/11°C and 27/24°C regimes, responded as did other species: under the latter conditions, plants were taller, had greater dry weights, fewer axillary branches, and larger total leaf areas.

TABLE 3

EFFECTS OF VARIOUS COMBINATIONS OF DAY AND NIGHT TEMPERATURE UPON GROWTH (AFTER 88 DAYS) OF PLANTS OF EUCALYPTUS BLAKELYI*

Height (mm)

D† \ N†	6°C	16°C	19°C	24°C
11°C	—	34	49 —	46
20°C	6	62 —	62	82
24°C	23	80 —	87	103
28°C	—	94	117	144

Weight of Stems (mg/plant)

D \ N	6°C	16°C	19°C	24°C
11°C	—	15	26 —	22
20°C	3	33 —	32	51
24°C	12	60 —	70 —	79
28°C	—	62	83 —	113

Weight of Leaves (mg/plant)

D \ N	6°C	16°C	19°C	24°C
11°C	—	79	118 —	101
20°C	9	176 —	150	246
24°C	47	298 —	335 —	351
28°C	—	240 —	319	452

Weight of Roots (mg/plant)

D \ N	6°C	16°C	19°C	24°C
11°C	—	72 —	86	38
20°C	5	109 —	102	135
24°C	28	174	227 —	176
28°C	—	134	153	202

Total Weight (mg/plant)

D \ N	6°C	16°C	19°C	24°C
11°C	—	167	230	160
20°C	16	321 —	286	445
24°C	86	532 —	632 —	606
28°C	—	436 —	554	767

Leaf Area (sq. cm.)

D \ N	6°C	16°C	19°C	24°C
11°C	(7)	133	224 —	278
20°C	12	295 —	279	471
24°C	76	445 —	528 —	594
28°C	—	408	562 —	696

* A non-significant ($P > 0{\cdot}05$) difference between two adjacent mean values is indicated by a line between them.

† D = average day temperature; N = average night temperature.

TABLE 3 (*Continued*)

	Branch Number				Branch Weight × 100/Top Weight				
D \ N	6°C	16°C	19°C	24°C	D \ N	6°C	16°C	19°C	24°C
11°C	—	4·4	3·5	6·7	11°C	—	23·9	19·5	36·0
20°C	2·7	4·2	3·6	3·5	20°C	11·0	16·6	8·3	11·0
24°C	5·5	4·9	3·8	1·4	24°C	25·4	15·9	11·2	1·5
28°C	—	3·1	1·0	1·0	28°C	—	8·0	0·7	0·6

Placing the species in order of dry weight increase, but not of height increase, over the period of 60 days, the sequence would appear to be *E. bicostata* > *E. blakelyi* > *E. polyanthemos* > *E. niphophila*, under comparable growing conditions.

Leaf form, like apical dominance, was of morphological interest. Figure 1 illustrates the leaf (third up from the cotyledons in *E. bicostata* and *E. polyanthemos*, fourth in *E. blakelyi*) having an area and shape nearest the average as determined by measurements of the comparable leaf on all plants of each species. In this way it was hoped to illustrate the effects of environment not only upon length, breadth, and area of leaf, but also on the many other parameters necessary for an adequate definition of leaf shape in species of *Eucalyptus*, the assumption being that leaves of average length, breadth, and area would also be average as regards such other parameters. Analyses of variance demonstrated that leaf shape (as measured by length/breadth ratio) and area were significantly different under the conditions in the various glasshouses, but the trends were not always in the same direction for each species.

(ii) *Experiment* 2.—Tables 3 and 4 include the results obtained with *E. blakelyi* (harvested after 88 days) and *E. pauciflora* (harvested after 58 days) grown under 16 combinations of day and night temperature. The results obtained with *E. stellulata*, used in a pilot experiment, did no more than indicate the general levels of response to day and night temperature likely to occur since the number of plants in many treatments was small and the plants were extremely variable. They duplicated, for the most part, the trends outlined for *E. pauciflora* below rather than those for *E. blakelyi*.

Considering the results for *E. blakelyi*, the two missing values arose because the plants died, deaths taking place earlier under the 28/6°C than the 11/6°C regime.

Shortest plants, of smallest stem, root, and leaf weight and, therefore, of total weight, and smallest leaf area, but not of smallest axillary branch number, occurred under the 20/6°C and the 24/6°C regimes, the former regime being more unfavourable for growth. Evidently the higher average day temperature in the latter case

helped to offset the retarding effect upon growth of the low night temperatures. But there was an optimum day temperature, plants under the 28/6°C regime dying after 48 days.

There was, similarly, an optimum night temperature when the average day temperature is 11°C. Growth was significantly increased when the night temperature rose from 16°C to 19°C. Further increase in night temperature to 24°C, however, while not significantly affecting height, total leaf area, and branch number, caused a significant reduction in total plant weight. This reduction appeared to be due largely to the reduction in weight of roots, a consequence perhaps of the fact that leaves of plants under the 11/24°C regime were noticeably chlorotic.

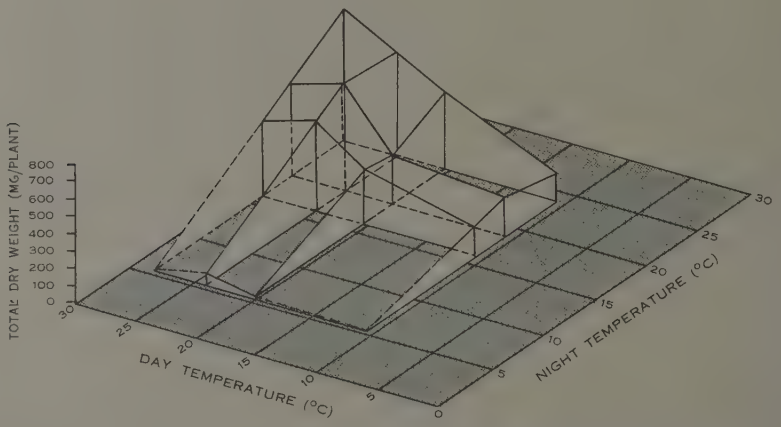

Fig. 2.—Three-dimensional diagram to illustrate the combined effect of day and night temperature upon total dry weight of plants of *Eucalyptus blakelyi*.

Increase in night temperature from 6°C to 24°C when the day temperature was either 20 or 24°C generally brought about increase in growth, though the increase in night temperature from 16°C to 19°C appeared to have little effect. Plants growing under the 24/24°C regime were significantly taller and had fewer branches than those under the 24/19°C regime.

In certain respects, plants under the 28/19°C regime differed significantly from those under the 28/16°C conditions. If the night temperature was increased further to 24°C, plant height, leaf and root weights, and total weight were increased significantly while branch number, leaf area, and stem weight were unchanged.

Irrespective of whether the average night temperature is 16, 19, or 24°C, increase in day temperature from 11°C to 24°C increased growth. Increase in day temperature to 28°C, however, while causing a significant increase in height, reduced root weight and axillary branch number. Tallest plants of highest total weight, largest total leaf area, and fewest axillary branches were produced under the 28/24°C regime. Such plants, however, except in height, did not differ significantly from plants growing under the 24/24°C regime. Plant height was apparently the most sensitive indicator of temperature changes.

Analyses of variance confirmed that the interaction between day and night temperature on growth was statistically significant. Such interactions are best illustrated by three-dimensional diagrams such as that shown in Figure 2. Irrespective of whether dry weight of leaves, stems, or roots, or total dry weight is plotted (here it is total dry weight), the form of the graph is more or less the same.

As indicated above, tallest plants tended to produce fewest branches. In other words, apical dominance found its highest expression under the temperature regimes 28/19°C, 28/24°C, and 24/24°C. But though absolute level of temperature was no doubt important in determining the weight of branches relative to the weight of tops and also the number of branches, there were indications that the magnitude of diurnal temperature fluctuations (temperature range) was also important in this respect. For example, highest branch numbers were recorded for plants growing under the 11/24°C (range 13°C) and 24/6°C (range 18°C) regimes. But the situation is by no means clear: undoubtedly, we are confounding effects of absolute levels of temperature with temperature range effects. Until we can carry out experiments in which these variables can be controlled, no conclusive statement as to their relative importance in the control of lateral branching is possible.

Comparison of the data for *Eucalyptus pauciflora* with those for *E. blakelyi* shows certain similarities as well as certain differences to exist in their growth response to various temperature regimes. Note that there was a considerable increase in the average day and night temperatures prevailing outside during the 58 days of the *E. pauciflora* experiment over those prevailing during the 88 days of the *E. blakelyi* experiment. When comparing the significance levels of mean differences between the species, it must be borne in mind that such levels are determined by the degree of intra-species variability. The statistical indications are that the variability of plants of *E. pauciflora* is greater than that of *E. blakelyi*. It is well to recall also the limitations of temperature control which underlie the small differences in average temperatures between comparable glasshouses during the two experiments.

In view of these remarks, it only appears worth while here to compare in a general way the results for *E. pauciflora* with those for *E. blakelyi*. The following points were noted:

(1) Like plants of *E. blakelyi* growing under conditions where the average night temperature considerably exceeds a relatively low day temperature, plants of *E. pauciflora* under the 14/24°C regime exhibited considerable chlorosis.

(2) The results obtained for *E. pauciflora* under the 27/20°C regime appeared anomalous in some respects (height, for example) in view of the otherwise consistent tendency for growth to increase with increasing day temperature irrespective of the prevailing night temperature.

(3) The facts that growth of *E. pauciflora* under the 20/20°C and 20/24°C regimes was not significantly different, and that stem weight and height were less under the 25/24°C than the 25/20°C regime, suggested that *E. pauciflora* did not possess the ability of *E. blakelyi* to respond by increased growth to higher night temperatures when day temperature was 20 or 25°C.

TABLE 4

EFFECTS OF VARIOUS COMBINATIONS OF DAY AND NIGHT TEMPERATURE UPON GROWTH (AFTER 58 DAYS) OF PLANTS OF EUCALYPTUS PAUCIFLORA*

Height (mm)

D† \ N†	8°C	17°C	20°C	24°C
14°C	10	24	31 —	30
20°C	19	47 —	46 —	42
25°C	28	46	74	56
27°C	36	75	61	75

Weight of Stems (mg/plant)

D \ N	8°C	17°C	20°C	24°C
14°C	6	21	35 —	28
20°C	12	40 —	40 —	34
25°C	21	39	74	49
27°C	27	55 —	49	68

Weight of Leaves (mg/plant)

D \ N	8°C	17°C	20°C	24°C
14°C	75	184	352 —	299
20°C	121	426 —	428 —	438
25°C	225	399	704 —	614
27°C	260	433 —	497	712

Weight of Roots (mg/plant)

D \ N	8°C	17°C	20°C	24°C
14°C	24	72	140	74
20°C	42	160 —	161 —	207
25°C	86 —	125	233 —	252
27°C	137 —	131 —	183	282

Total Weight (mg/plant)

D \ N	8°C	17°C	20°C	24°C
14°C	104	277	526 —	401
20°C	175	626 —	576 —	679
25°C	332	563	1012 —	915
27°C	425	619 —	728	1062

Leaf Area (sq. cm.)

D \ N	8°C	17°C	20°C	24°C
14°C	100	284	444 —	426
20°C	213	523 —	556 —	592
25°C	317	479	851 —	716
27°C	382	671 —	613	930

* A non-significant ($P > 0{\cdot}05$) difference between two adjacent mean values is indicated by a line between them.

† D = average day temperature; N = average night temperature.

TABLE 4 (*Continued*)

	Branch Number				Branch Weight × 100/Top Weight				
D \ N	8°C	17°C	20°C	24°C	D \ N	8°C	17°C	20°C	24°C
14°C	2·9 — 3·4 — 3·3 — 2·8				14°C	7·4	16·4	17·5	13·8
20°C	4·0	2·9	4·2	2·7	20°C	14·7	9·6	11·1	3·2
25°C	3·7 — 3·9		0·7 — 1·5		25°C	17·3	10·9	8·3	0·9
27°C	4·1 — 4·0		1·3 — 0·4		27°C	17·6	7·5	4·1	1·9

(4) As with *E. blakelyi*, tallest plants of *E. pauciflora* had fewest branches and occurred under the 27/20°C, 27/24°C, and 25/24°C regimes. However, the number of branches formed by plants under the 25/20°C regime was relatively small compared with *E. blakelyi*. But this difference between the species was not apparent when the ratio branch weight/tops weight was considered, low branch number apparently being compensated for in *E. pauciflora* by relatively large size of branches. Consideration of the values for this ratio brings out another point, evident also in the results for *E. blakelyi* and *E. stellulata*: values obtained for plants grown both day and night under glasshouse conditions were, for the most part, significantly less than those obtained for plants under regimes attained by placing the plants outside during either day or night. As in the case of branch number, we are led to suspect the influence of factors other than mean temperature levels, such as the magnitude of temperature fluctuations or rates of temperature change.

(5) Figure 3 illustrates differences in leaf size and shape which result when plants are grown under the different temperature regimes. The leaf shown is that corresponding most nearly to the average leaf size and shape as determined by measurement of the leaves of all plants under each regime. Analyses of variance indicated that shape (as measured by length/breadth ratio) was a function of the combined effect of day and night temperature, but was not related to either day or night temperature alone.

Finally, some consideration might be given to the values of the various ratios—T/R, L/S, and so on. The values may be calculated from the data in Tables 3 and 4. The ratios T/R and L/S varied respectively from 1·3 to 3·4 (average 2·3) and from 3·8 to 5·3 (average 4·5) for *E. blakelyi*. For *E. pauciflora*, values were higher—respectively 2·1 to 4·4 (average 3·0) and 9·6 to 12·9 (average 10·5)—this species apparently showing a greater tendency than *E. blakelyi* to accumulate dry weight in leaves rather than stems and in tops rather than roots. This conclusion can also be drawn from a consideration of the ratios of the weights of the various plant parts—stems, roots, and leaves—and of leaf area to total plant weight. Considering only

the regimes attained wholly under glasshouse conditions, in the case of *E. blakelyi*, values for the ratios *S*/total weight, *L*/total weight, *R*/total weight, and leaf area/

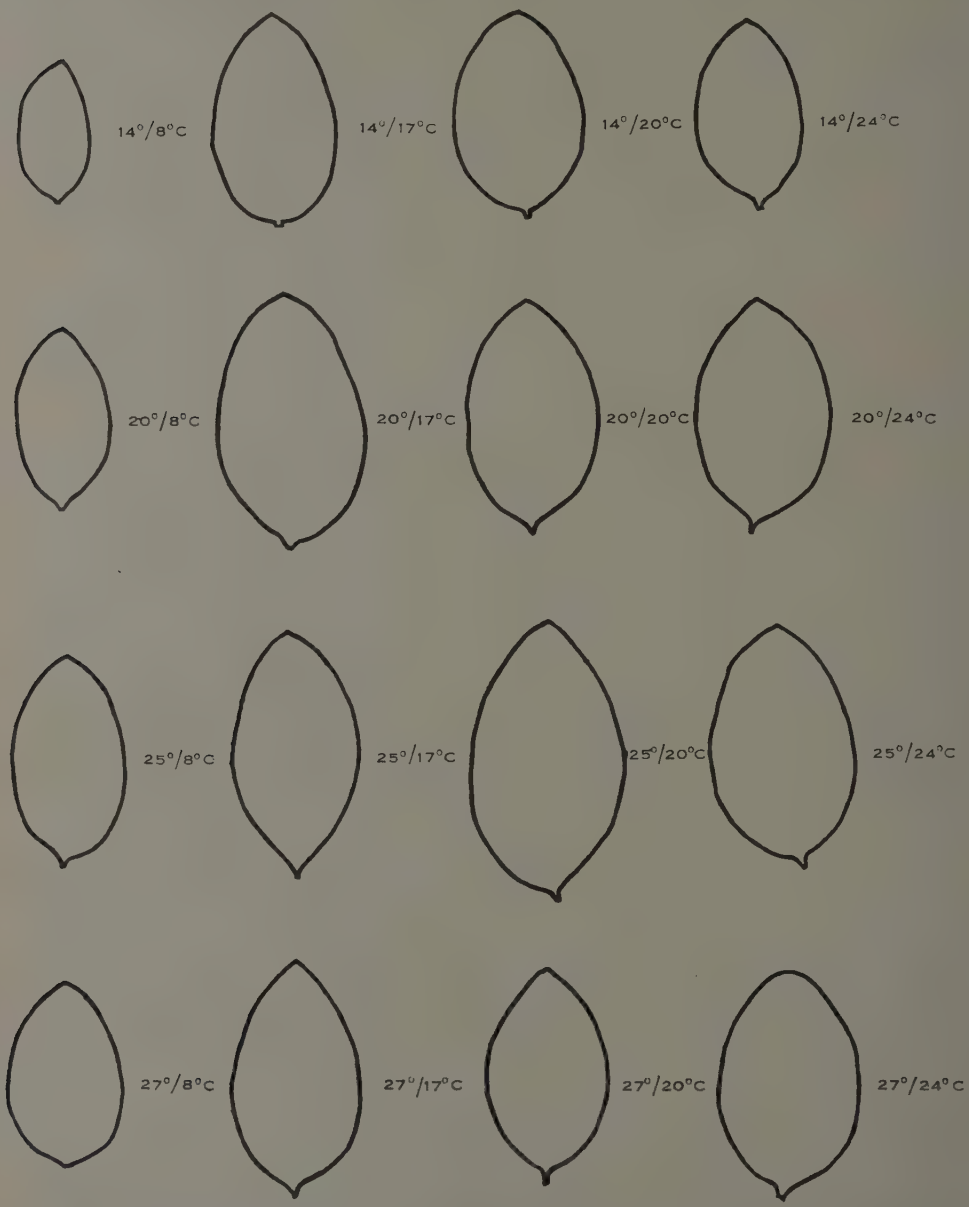

Fig. 3. –Effects of temperature on the shape of the second leaf of *Eucalyptus pauciflora*.

total weight were respectively 0·10–0·15, 0·53–0·59, 0·26–0·36, and 0·84–1·02. For *E. pauciflora*, the values were respectively 0·05–0·09, 0·64–0·71, 0·21–0·28, and 0·78–1·08.

Statistical treatment of ratio results has not been undertaken in view of the inconsistencies which occur. Casual inspection of the results indicates no obvious trends in the ratios of *L*/total weight or leaf area/total weight for either species; a reduction in *R*/total weight ratio appears to accompany increase in day temperature in both species; an increase in the ratio *S*/total weight with increase in day temperature appears to occur with *E. blakelyi*, but is not obtained consistently with *E. pauciflora*. Note, too, the high values of *L*/total weight and leaf area/total weight, and the low values of *R*/total weight under the 11/24°C and 14/24°C regimes respectively in *E. blakelyi* and *E. pauciflora*, the plants being chlorotic.

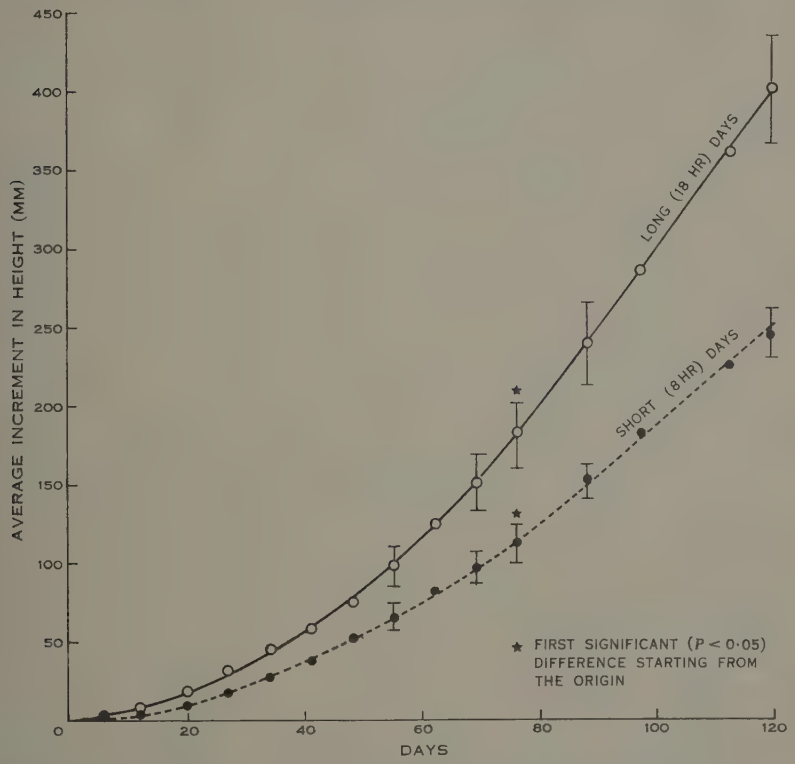

Fig. 4.—Effects of day length on height of plants of *Eucalyptus rubida*.

(*b*) *Day-length Experiments*

The response of all species to day length was the same as that of *E. rubida*, data for which are given in Figure 4. Increased day length, when temperatures were adequate for growth, evidently elicited an increased growth response. *Eucalyptus* species would thus belong to the relatively small Class C of Nitsch (1957): woody perennials in which short days do not bring about cessation of growth.

The effects of the two day-length treatments upon the shape of comparable leaves on the main axis are illustrated in Figure 5. Generally, under *SD*, leaves were darker green (owing to their higher total chlorophyll content and not to a changed

ratio of chlorophyll *a*/chlorophyll *b*) and, in *E. bicostata* especially, had undulating margins. *LD* were apparently more conducive than *SD* to the formation of leaves

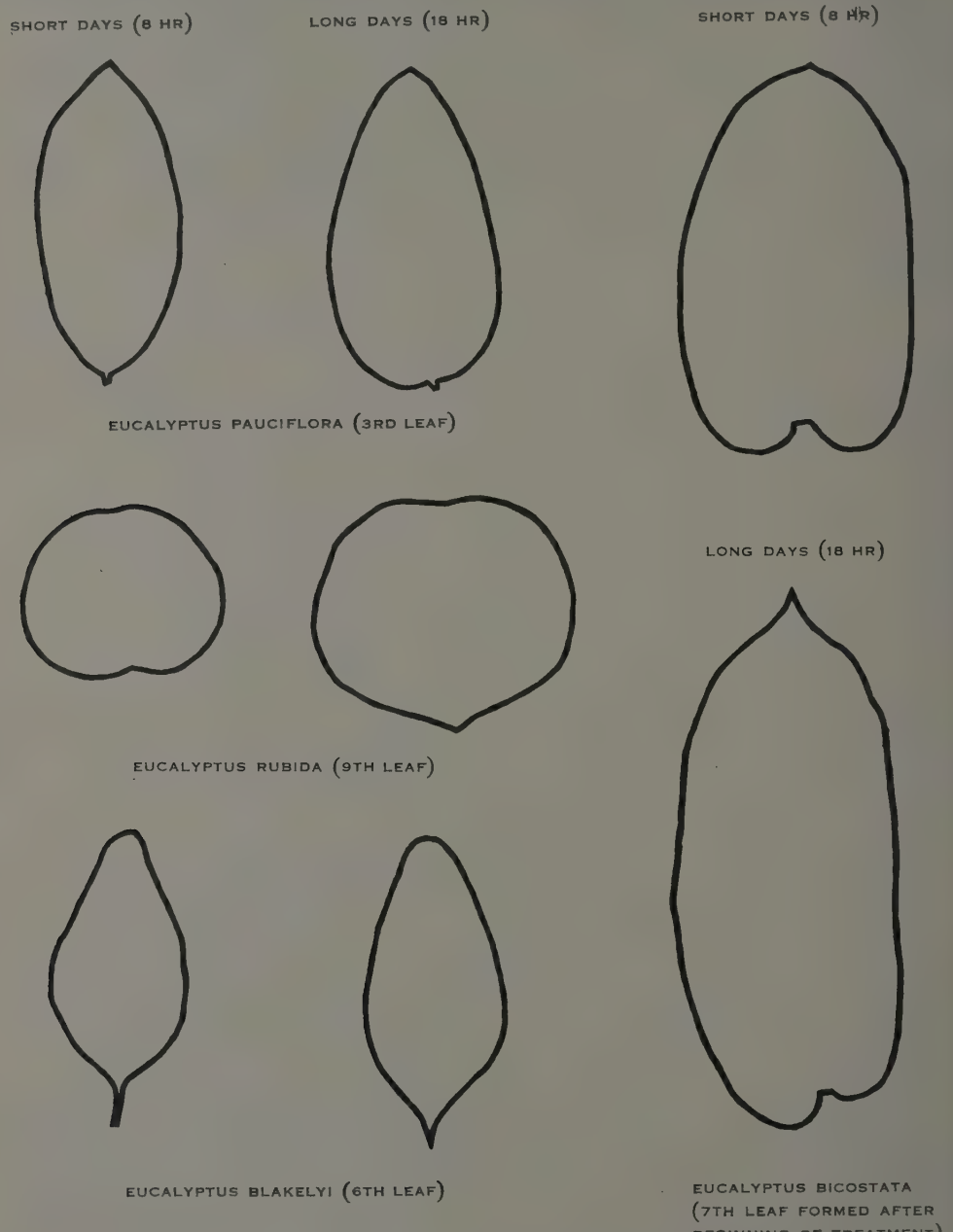

Fig. 5.—Effects of day length on leaf shape in species of *Eucalyptus*.

having more nearly the shape of adult leaves. In addition, plants under *LD* produced a significantly greater number of leaves (and of internodes); dry weights of leaves

and stems were significantly higher, and stem diameter was greater. Taking the results for *E. rubida* as an example: dry weights of leaves of *LD* plants were 1·8 times and of stems 2·0 times those of *SD* plants; stem diameters were 1·4 times those of *SD* plants.

(c) *Day Length–Temperature Experiments*

Only one transfer from outside conditions to the heated glasshouse was possible with *E. blakelyi* as the remainder of the plants outside were killed by frost some days afterwards. Five transfers of plants of *E. pauciflora* were made and data on the height growth of these plants after transfer are given in Figure 6.

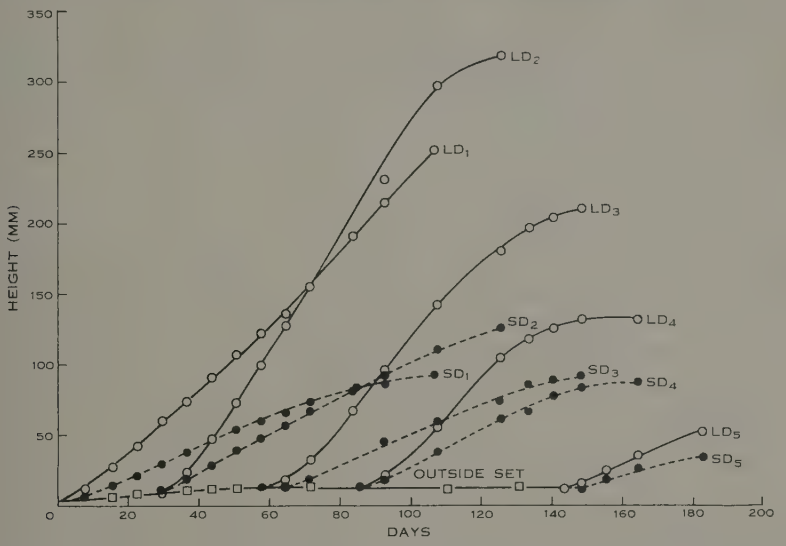

Fig. 6.—Effects of day length on height of seedlings of *Eucalyptus pauciflora* transferred to glasshouse conditions after various periods of time (indicated by numbers 1–5) under outside conditions.

Control plants of either species which remained outside grew scarcely or not at all. Exposure of plants of *E. blakelyi* to winter conditions for 48 days (April 16–June 3) reduced their rate of growth when they were transferred to higher temperatures and either *LD* or *SD* in the glasshouse. Plants of *E. pauciflora*, however, after remaining outside for 28 days (April 16–May 14), not only showed an enhanced growth rate over plants not so exposed, but also an increased difference between the *SD* and *LD* response curves. Growth rates of plants transferred on subsequent occasions to *SD* and *LD* conditions diminished with each successive transfer, as did also the difference between the *SD* and *LD* response curves. Verification of these effects of low-temperature treatment in the case of *E. pauciflora* is necessary before firm conclusions can be drawn: the enhanced growth rate of plants transferred on the first and, possibly, second occasions, for example, might well have been due to chance, bearing in mind the absence of a similar response in *E. blakelyi*. But despite this, there appears to be no doubt that the length of the period of low-temperature

(winter) treatment can affect the rate of growth when this is resumed under suitable temperature conditions as well as determine the magnitude of the difference in height which increase in day length from 8 to 16 hr can then bring about.

IV. Discussion

When considering the results above, the extent to which it was possible to control the environmental factors should be borne in mind. Despite the limitations in the degree of environmental control, however, the various environmental regimes were sufficiently different to enable us to obtain some indication of the temperature and day-length tolerances of the various species of *Eucalyptus* examined and to relate these to the gross morphological characteristics and performance of such species. It does not appear profitable, however, to attempt a detailed physiological interpretation of the intra-species responses to various temperature regimes, more especially when we consider that nothing is known about the more specific effects of temperature upon species of *Eucalyptus*, even upon such overall processes as photosynthesis, respiration, and translocation.

In the cases of *E. bicostata*, *E. blakelyi*, *E. pauciflora*, and *E. polyanthemos* under *SD*, day temperatures of 24–30°C with night temperatures 3–5°C lower approach optimal conditions for growth (Tables 1, 3, and 4). Increasing day length from 8 to 18 hr increases growth. It might thus be anticipated that climates of relatively high mean annual temperature, equable in the sense that seasonal fluctuations of mean temperature and diurnal temperature fluctuations are relatively small, combined with days which are relatively long, would be most nearly optimal for growth in height and weight of seedlings of *Eucalyptus* species, provided other factors were non-limiting.

While the slowing down of growth of species of *Eucalyptus* with the approach of winter conditions appears to be a response to the combined effect of shortened days and reduction in day and night temperatures, final cessation of growth is a response to temperature. It is probable that the length of the period of exposure to winter conditions affects the rate of regrowth in the following season when favourable growing conditions return; likewise capability of the species to respond to lengthened days. The situation is similar to that which Kramer (1957) found in one-year-old seedlings of *Pinus taeda:* seedlings grown at relatively high temperatures resumed growth sooner and made more growth early in the season than those grown at lower temperatures.

Species of *Eucalyptus* differ from *Pinus taeda* (Kramer 1957) and *P. lambertiana* (Hellmers 1959) in their response to different combinations of day and night temperatures. Kramer found with *Pinus taeda* that when the night temperature was maintained at 17°C, the amount of shoot growth tripled as the day temperature was increased from 17°C to 30°C, but when the day temperature was maintained at 23°C, the amount of shoot growth was reduced by 50% by increasing the night temperature from 11°C to 23°C. Best growth was made when the difference between day and night temperatures was widest (12°C or 13°C) and poorest growth when nights were as warm as days. Hellmers grew one-month-old seedlings of *Pinus lambertiana* for 6

months under 16-hr days with night temperatures of 4°C or 17°C combined with a series of day temperatures, and with day temperatures of 10°C or 23°C combined with a series of night temperatures. Optimum temperatures for growth were a day temperature of approximately 23°C combined with a less critical night temperature of between 4°C and 17°C. Night temperatures above 10°C combined with a 10°C day temperature increased dry weight production, but lower night temperature had no effect.

Regarding species distribution, the similarity (in so far as it has been established here) of temperature and day-length regimes for optimum growth of species of *Eucalyptus* as diverse in geographical range as, for example, *E. blakelyi* and *E. pauciflora* calls for comment. It would suggest that, in so far as temperature and day length are concerned, distribution probably depends more upon the temperature and day length tolerance ranges (the adaptability) of the species in question than upon differences in performance under regimes approaching the optimal. Thus, de Beuzeville (Forestry Commission of New South Wales 1943), describing the distribution of forest species in New South Wales, gives the mean temperature of the coldest month in areas occupied by *E. niphophila*, *E. stellulata*, and *E. pauciflora* as −1–6°C, by *E. bicostata* as 1–8°C, and by *E. blakelyi* and *E. polyanthemos* as 6–9°C. Though the data here were inadequate for a comparison of the relative tolerances of seedlings of these species to low temperatures, there was no doubt that seedlings of *E. pauciflora* could withstand outside winter conditions (at Canberra, A.C.T.) better than those of *E. blakelyi* (Section III (*c*)). The performance of *E. bicostata* was better than that of *E. blakelyi* and that of *E. blakelyi* better than that of *E. polyanthemos* under an outside 14/8°C regime (Table 1).

As it is growth under the *SD* of winter with which we are dealing, consideration of the relevance of the data to species distribution in relation to the mean temperature of the hottest month in areas where the species occur and the difference (temperature range) between this and the temperature of the coldest month, after the manner of Swain (1939) and de Beuzeville (Forestry Commission of New South Wales 1943), appears premature. All that can be said at the moment regarding the relative tolerance of the species to higher temperatures is that *E. polyanthemos* was the only species which grew significantly less actively under the 27/24°C than the 25/20°C regime.

It might be mentioned in passing that Swain regarded the seasonal distribution of rainfall as the most important climatic factor influencing the distribution of tree species in Australia.

The data serve to emphasize the point that whilst it is relatively easy to determine the environmental optima for performance, the determination of environmental tolerance ranges, the prediction of their influence on distribution, and of the effects of their interplay with other factors, are problems considerably more complex. It would appear that the easiest approach to such problems is by way of extensive transplant experiments into environments carefully measured and defined over a time period relatively long in comparison with the life span of the species investigated.

We might bear in mind also that where natural, as opposed to man-made, distributions are being considered, some consideration must be given to the effects of other factors, such as immigration opportunity.

V. Acknowledgments

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MYCORRHIZA IN THE NEW ZEALAND ERICALES

By R. F. R. McNabb*

[*Manuscript received November* 2, 1960]

Summary

The New Zealand species of the Ericaceae and Epacridaceae examined possessed mycorrhizas anatomically similar to those of *Calluna* and *Vaccinium*.

The endophyte of *Pernettya macrostigma* was isolated and successfully back inoculated into the host, under controlled conditions. Its taxonomic identity was uncertain because of its reproductive sterility.

A fungus indistinguishable from the ericaceous endophyte was found to form mycorrhizas with Dicotyledons other than the Ericales.

I. Introduction

The New Zealand Ericales are mostly small prostrate or erect shrubs except for some species of *Dracophyllum* which may reach 20 ft in height. They belong to the Ericaceae and Epacridaceae.

In New Zealand, endemic species of the Ericaceae have been shown to possess mycorrhizas typical of the family (Brook 1952). References to the mycorrhizas of the Epacridaceae are few. In Australia, McLennan (1935) worked with *Epacris impressa* and referred to an earlier unpublished work by Baron on the mycorrhizas of other species of the Epacridaceae. Both McLennan and Baron found that the mycorrhiza of *Epacris impressa* agreed closely with that of *Calluna vulgaris*. Burges (1936) recorded the presence of mycorrhizas in Australian Epacridaceae.

In the present investigation the following species were examined:

Ericaceae.—*Pernettya macrostigma* Col., *Gaultheria antipoda* Forst., *G. rupestris* R. Br.

Epacridaceae.—*Cyathodes colensoi* Hook. f., *C. empetrifolia* Hook. f., *C. pumila* Hook. f., *Dracophyllum prostratum* Kirk, *D. longifolium* R. Br., *Leucopogon fraseri* A. Cunn., *Pentachondra pumila* R. Br.

Successful attempts were also made to establish a pure culture of the endophyte from *Pernettya macrostigma*.

II. Morphology of the Mycorrhiza

All the species examined possessed a root system which ultimately branched into fine white rootlets, 50–100 μ in diameter. The amount of branching and length of the fine rootlets were not as great in the Epacridaceae as in the Ericaceae.

Anatomically, the rootlets were similar in all species, consisting of a small stele, surrounded by an endodermal and a cortical layer, each one cell thick. Root hairs and piliferous layers were absent.

* Plant Diseases Division, D.S.I.R., Auckland, New Zealand.

The fine rootlets were normally converted to mycorrhizas and initial infection took place at any point along the root length. As the endophyte was normally abundant on the root surface, it was usual for the more recent infections to be nearer the root tip and the older infections further removed, but in growing roots the apical meristem and elongating region remained free from the endophyte. Non-meristematic root tips were often infected.

In the early stages of mycorrhiza formation, a network of fine young hyaline hyphae, 0·5–0·8 μ in diameter, spread over the root surface. Fine hyphal branches penetrated the cortical cells through their outer walls, filling each with a dense coil. Infection from an adjacent cell was rare.

With maturity, walls of the external hyphae thickened and septa became visible. Mature external hyphae were 1·5–2·2 μ in diameter. After death, external hyphae became dark brown, their segments often inflated and irregular in shape, and formed empty chains which persisted indefinitely as part of the network of surface hyphae.

As the internal hyphae matured, their walls thickened and the infected cells appeared granular. At maturity, the intracellular mycelium differed from the external mycelium in having fewer and more irregularly spaced septa, a more irregular outline, and a greater diameter—approximately 1·6 times that of the external hyphae. Mature intracellular hyphae contained globules which gave a positive reaction for fats.

Intercellular hyphae were rarely present.

In older infections, the moribund intracellular hyphae, although devoid of contents, remained for an indefinite period as groups or chains of inflated segments. They resembled old segments of the external mycelium but were more regular in outline. Alternatively, in some cells hyphal clumps were formed in which the individual hyphae and their contents underwent a gradual conversion to a structureless mass which tended to surround the enlarged cell nucleus. In the final stages of disintegration, hyphal masses were completely digested by the activity of the host cells, leaving the cell nuclei slightly enlarged. A similar sequence was described by Rayner (1927).

In all species there was no penetration of the stelar tissue.

Brook (1952) investigated the endophyte of *Pernettya macrostigma* and described the formation of spore-like sacs by the endophyte within the cortical cells of older infected rootlets. Morrison (1954) found these bodies, which he interpreted as sporangia, on the surface of the rootlets as well as within the cortical cells. Both internal and external sporangia were found in the course of this investigation, but only on the rootlets of *P. macrostigma*.

The external sporangia were thin-walled, spherical to ovoid bodies, ranging from 2 μ to 6 μ when fully expanded, borne on sporangiophores which appeared to arise monopodially from thin-walled hyphae attached to the surface mycelium. Within the rootlets, sporangia numbered up to 20 per cell, and in such cases it was impossible to distinguish sporangiophores for the sporangia occupied almost the entire cell.

P. macrostigma was the only species in which sporangia were found, and they only occurred on rootlets of potted plants grown in heath soil, never in specimens taken from their natural habitats. Morrison (1954) also found sporangia only on roots of seedlings potted in heath soil to which additions of nitrogen and phosphorus had been made. No other species was studied in pot culture.

Apart from the Ericales, 7% of the Dicotyledons examined possessed a mycorrhizal fungus indistinguishable from the ericaceous endophyte, viz. *Cassinia fulvida* Hook. f. (Compositae), *Coprosma propinqua* A. Cunn. (Rubiaceae), and *Nothopanax colensoi* Seem. (Araliaceae). This indicated that the endophyte was not dependent solely upon the Ericales and that it was widely spread in natural soils. In the roots of *Nothopanax colensoi* the endophyte formed the same type of inflated, brown segments within the epidermal cells as did the typical ericaceous endophyte. Brook (1952) found that a fungus capable of forming a typical mycorrhiza with *Pernettya macrostigma* was present in forest soils where no Ericales grew.

III. Isolation of the Endophyte

Pernettya macrostigma was chosen as the host species and roots were taken from potted plants growing in heath soil to which NaH_2PO_4 (1·5 g per 3½ in. pot) had been added six months previously, since Morrison (1957) found that a heavy dosage of phosphorus in this form caused an inhibition in root growth in *P. macrostigma* seedlings but increased the intensity of mycorrhizal infection.

A centrifuge method previously employed by Hopkins (1954) was used to free the mycorrhizal roots from surface contaminants. Earlier attempted isolations (Brook 1952) showed that chemical surface sterilants were unsatisfactory because of the fineness of the rootlets, and the fact that the endophyte was confined to a single layer of superficial cells. Chemical sterilants killed the internal as well as the external mycelium.

The centrifuging apparatus consisted of a small glass tube with a piece of fine muslin stretched over one end and kept in place with waterproof adhesive tape. The base of the tube was wrapped in cotton wool and the whole tube fitted into a centrifuge tube. Sufficient sterile water was added to cover the muslin on the top of the inner tube.

Selected rootlets were brushed and washed in running tap water for three hours before centrifuging.

The rootlets were placed on the muslin and centrifuged at 4500 r.p.m. (*c.* 2250 g) for 10 min before being removed and shaken in two changes of sterile water. This process was repeated three times and after the final centrifuging, the rootlets were shaken in four changes of sterile water. Prior to each centrifuging, the apparatus was sterilized with 50% alcohol and flushed with two changes of sterile water.

After the final shaking, the rootlets were cut into 2–4 mm lengths and inoculated onto plates of Burgeff's medium (Wolf 1954). The plates were incubated at 25°C.

After three weeks, 67% of the plates were sterile, 20% were contaminated with common moulds, and 13% showed a slow-growing fungus appearing from the inocu-

lum. This fungus was subcultured on to plates and tubes of Burgeff's medium where it continued to grow at a slow rate.

IV. Synthesis of the Mycorrhiza

Brook (1952) showed that *Pernettya macrostigma* was capable of aseptic growth on artificial media. Satisfactory germination and growth were obtained using cotton wool moistened with heath soil extract, but the lateral branches of the root system were usually suppressed. In this present investigation, seedlings were grown on a semi-liquid agar (Bain 1937).

Seeds of *P. macrostigma* were removed from fruits which had been left in the open during the winter, and surface sterilized with calcium hypochlorite solution (Brook 1952). They were then transferred directly to tubes of heath soil extract solidified with 0·4% agar. The extract was prepared by standing heath soil in an equal volume of water for 24 hr before decanting off the supernatant liquid. Agar was added and the whole autoclaved for 20 min at 15 lb pressure.

After six weeks 60% of the seeds had germinated. To each of 10 tubes containing a germinated seedling, a piece of agar containing the fungus was added as a source of inoculum, and the tube replugged and capped with plastic. The control tubes received the same treatment as the rest, without the addition of inoculum. After nine months the seedlings averaged 3·0 cm in height with rather sparse root systems, the main roots of which were up to 2·5 cm long. No plants showed the suppression of laterals figured by Brook (1952). At this stage the experiment was taken down and the roots examined.

The 4 control seedlings were sterile. Of the 10 inoculated tubes, the germinated seedlings failed to continue growth in 4, but in the remaining 6 tubes the fungus synthesized the typical ericaceous mycorrhiza with the roots. A second experiment of similar design confirmed this result, but no mycorrhizas could be detected in an experiment dismantled after a lapse of five months from inoculation.

V. Cultural Characteristics of the Mycorrhizal Fungus

Mature colonies were sterile, dark reddish brown to fuscous black almost to the margins, and appeared to be self-limiting on all media used, e.g. potato dextrose, malt and prune agars, Burgeff's, McArdle's, and Czapek media, and a modification of a complex medium used by Winkberg and Fries (1952). The mycelium was mainly submerged but aerial tufts of pale, dull grey hyphae sometimes tinged with olive grey were produced in the centre of the colonies.

On Burgeff's medium, hyphae were even in diameter (1·5–2·0 μ), rather regularly septate, with hyaline walls which later became pallid brown. With age, hyphae became irregularly septate, often with rounded or irregularly inflated segments, and the walls became dark brown.

The most prominent characteristic of the fungus was the slow rate of growth, amounting to *c.* 0·1 mm per day at 25°C. Because of this, no attempt was made to explore its cultural reactions in detail.

The fungus agreed closely in rate of growth, colour of mycelium, diameter and septation of hyphae, and lack of fruiting bodies with *Mycelia radicis myrtillii* α (Freisleben 1934). Bain (1937) has also reported extremely slow growth rates for endophytes isolated by him from *Ledum*, *Chamaedaphne*, and *Vaccinium*.

VI. Acknowledgments

Most of this investigation was carried out at the Botany Department of the University of Otago. The author is indebted to Professor G. T. S. Baylis and Dr. G. P. Harris of that Department for their helpful advice and criticism throughout the course of this work.

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CYTOTAXONOMY OF AUSTRALIAN *DANTHONIA*

By R. D. Brock* and J. A. M. Brown*†

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Summary

Cytological and cytogenetic studies of 29 Australian species of *Danthonia* revealed an extensive polyploid series ranging from $2n = 24$ to $2n = 120$. Meiosis was regular and fertility high in the species. Field hybrids were detected by aberrant chromosome number, meiotic behaviour, and reduced fertility.

An extensive series of interspecific crosses resulted in some viable hybrids but many combinations failed or gave F_1 plants which failed to flower. Most viable F_1 plants were highly sterile. Diploid hybrids were completely sterile, but in higher polyploids crossing was more often successful and hybrids had increased fertility.

Meiotic pairing and chiasma frequency were drastically reduced in all F_1s. Multivalent associations indicated gross structural rearrangements. The restoration of fertility by chromosome doubling indicated cryptic structural changes.

Neither cross compatibility nor meiotic behaviour showed any strong relationship with taxonomic grouping.

These results together with the intermediate morphology of F_1s and the increased morphological variation in polyploid forms of *D. caespitosa* and *D. linkii* strongly suggest alloploidy as the main mechanism of evolution of the polyploid species.

Twenty-three of the species had Panicoid type of epidermis and Festucoid chloroplast arrangement. The exceptions were *D. pallida*, *D. procera*, *D. frigida*, *D. nivicola*, *D. nudiflora*, and *D.* sp. indescr.

These studies throw light on the relationship of the arid form, *D. bipartita*, the alpine forms *D. frigida*, *D. nivicola*, and *D. nudiflora*, and the highly variable forms such as *D. caespitosa* and *D. linkii* with the rest of the genus.

Plant improvement by conventional methods would be virtually impossible unless restricted to high polyploids and intraspecific crossing, but true-breeding, highly fertile amphidiploids could be produced.

I. Introduction

The genus *Danthonia* DC. is widely distributed throughout the temperate regions of both the northern and southern hemispheres. In Australia many species are found in the southern part of the continent but few occur in the subtropical regions and only *D. bipartita* is found in the more arid region.

The genus is of economic importance over vast areas of natural grassland in eastern, southern, and western temperate regions. Although various species received attention from ecologists and agronomists (Breakwell 1923; Trumble and Davies 1931; Richardson, Trumble, and Shapter 1931; Cashmore 1932) there was considerable taxonomic confusion until the recent revision of the genus by Vickery (1956).

* Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T.

† Present address: Biology Department, Brookhaven National Laboratory, Upton, Long Island, N.Y., U.S.A.

Vickery described a number of new species and revised others. She used the existence of discontinuity as the main species criterion but pointed out that "not all biological populations showed discontinuity". Consequently her revision is based on "a compromise which accepts practical needs as of vital importance at the present stage of our knowledge of the genus, and falls short of the ideal of absolute discontinuity". This revision utilized only the morphological characters and geographic distribution of the specimens examined. Subsequently Abele (1959) published chromosome numbers of a range of *Danthonia* species. The work reported here extends this study of chromosome numbers and attempts to assess the evolutionary pattern and relationships between the species by the ease of hybridization, growth, and fertility of the F_1 hybrids and the chromosomal relationships as revealed in these hybrids.

II. Material and Methods

Seed and clonal material collected in the field, and seeds obtained from the Waite Agricultural Research Institute, together representing collections from all States of Australia, were grown under glasshouse conditions and chromosome numbers were determined. Herbarium specimens have been placed in the Herbarium of the Division of Plant Industry, C.S.I.R.O., Canberra. All taxonomic classifications were made on the basis of Vickery's (1956) taxonomic revision.

One species not described by Vickery, *D.* sp. indescr., has been included. A description of this species will be published shortly (M. Gray, personal communication). Another species, aff. *D. alpicola*, does not exactly fit the description of *D. alpicola* J. Vickery. This species bears a relationship to *D. eriantha* similar to that which *D. alpicola* J. Vickery bears to *D. caespitosa* (Vickery, personal communication).

Chromosome counts were made from root tips after pretreatment in α-bromonaphthalene, fixation in 1 : 3 acetic alcohol, 12 min hydrolysis in N.HCl at 60°C, and staining in Feulgen solution. Squashes were made in 0·1% acetic orcein. Meiosis was examined in squash preparations after fixation in Carnoy's fixative (Darlington and La Cour 1960) or Bradley's (1948) fixative. Fixed material was stored at −14°C until required for examination.

Pollen fertility was assessed by staining pollen at or near the time of dehiscence with 1% cotton blue in lactophenol. Because of the variable number of florets in a spikelet, recording of percentage seed set (Tables 6 and 7) was restricted to two basal florets per spikelet, and hybridization was attempted only with basal florets.

Controlled hybridizations were made during two successive years. A few species of *Danthonia* are cleistogamous but in most of the species the florets open for a short period during either the early morning or late afternoon. Where possible, emasculation and pollination were done when the florets opened naturally. Although a late summer–early autumn flowering period occurred in both years, this was erratic, and crossing was restricted to the normal spring–early summer flowering.

Results of hybridizations were recorded as empty florets, aborted seeds, small or shrivelled seeds, or plump seeds. Germination of seeds was attempted under sterile conditions after 5 days' cold treatment (5°C). Examination of leaf anatomy was made following the methods described by Prat (1948).

TABLE 1

CHROMOSOME NUMBER OF DANTHONIA SPECIES*

<table>
<tr><th>2n = 24</th><th>2n = 48</th><th>2n = 72</th><th>2n = 96</th><th>2n = 120</th><th>2n = 42</th></tr>
<tr><td rowspan="3">D. acerosa (2)
aff. D. alpicola (5)
D. auriculata (4)
D. carphoides (4)
D. duttoniana (4)
D. monticola (1)
D. occidentalis (1)
D. penicillata (21)
D. pilosa (4)
D. racemosa and var. (15)
D. semiannularis (1)
D. setacea (9)
D. nudiflora (9)
D. sp. indescr. (1)</td><td>D. clelandii (1)
D. eriantha (10)
D. geniculata (1)
D. richardsonii (8)
D. setacea var. breviseta (3)</td><td>D. bipartita (6)
D. purpurascens (10)</td><td>D. procera (1)</td><td></td><td rowspan="4">D. frigida (5)</td></tr>
<tr><td colspan="4">D. induta (4)</td></tr>
<tr><td colspan="2">D. nivicola (8)
D. longifolia (3)
D. pilosa var. paleacea (3)
D. linkii (15)</td><td colspan="2">D. pallida (3)</td></tr>
<tr><td></td><td>D. linkii var. fulva (11)</td><td></td><td></td></tr>
<tr><td colspan="3">D. caespitosa (28)
D. laevis (11)</td><td></td><td></td></tr>
</table>

* The number after the species name is the number of collections counted.

III. Results

(*a*) *Chromosome Numbers*

Table 1 summarizes the chromosome numbers of the 33 species or varieties examined. Details of collecting localities are given in Appendix I. Some of these chromosome counts are reported for the first time, others confirm the counts made by previous workers (Myers 1947; De Wet 1954*a*; Abele 1959). This is the most extensive series so far reported and it includes all numbers recorded for Australian species with the exception of $2n = 48$ for *D. bipartita* (De Wet 1954*a*). A polyploid series ranging from $2n = 24$ to $2n = 120$ exists within the Australian species of the genus. The Australian species of $2n = 24$ behave as functional diploids and throughout this paper they are referred to as diploids. However, four South African species have $2n = 12$ (De Wet 1960), indicating a basic number of $x = 6$ for the genus.

The genus *Danthonia* is phylogenetically very old (De Wet 1954*a*) and it appears that the only remaining ancestral diploids are restricted to South Africa. The Australian functional diploid species ($2n = 24$) are probably very old polyploids stabilized by an amphidiploid type of chromosome pairing.

With the exception of *D. frigida*, all of the Australian species examined are polyploids on the basic number $x = 6$. *D. frigida*, $2n = 42$, is, however, based on $x = 7$. This species is restricted in distribution to areas above 5000 ft altitude on Mt. Kosciusko. *D. frigida* has its morphological and ecological affinities with several New Zealand alpine forms such as *D. cunninghamii*, *D. raoulii*, and *D. flavescens* (Vickery 1956), which are also based on $x = 7$, suggesting that *D. frigida* is a relic species and that it is not closely related to the other Australian species of *Danthonia*.

D. bipartita is also aberrant morphologically, ecologically (Vickery 1956), and chromosomally (Plate 1). It has bulbous, woolly, basal leaf sheaths and it inhabits the arid regions of central and northern Australia where no other *Danthonia* species occur. Although it has $2n = 72$, which fits the general chromosomal polyploidy pattern of the other Australian *Danthonia* species, it has very much smaller chromosomes.

The remaining 27 species and 4 varieties form a more compact group with regard to their morphological, ecological, and chromosomal ranges. Nine of the species occur with more than one chromosome number. Five had two chromosome forms: *D. longifolia* (24, 48), *D. nivicola* (24, 48), *D. pallida* (72, 96), *D. pilosa* and its variety *paleacea* (24, 48), *D. setacea* and its variety *breviseta* (24, 48); and four have three chromosome forms: *D. caespitosa* (24, 48, 72), *D. induta* (72, 96, 120), *D. laevis* (24, 48, 72), and *D. linkii* and its variety *fulva* (24, 48, 72). The remaining 18 species have so far only been found as single chromosome number forms, either $2n = 24$, 48, 72, or 96. In several of these species the number of different collections examined is small and the possibility of even more extensive polyploidy is not excluded.

(*b*) *Chromosome Number and Taxonomic Grouping*

The main taxonomic feature used to separate the Australian species is the arrangement of the hairs on the back of the lemma (Vickery 1956). By this criterion the species can be divided into three major groups. Group A has hairs scattered

TABLE 2

RELATIONSHIP BETWEEN TAXONOMIC GROUPING AND CHROMOSOME NUMBER OF DANTHONIA SPECIES

Group	Lemma Hairs	$2n = 24$	$2n = 48$	$2n = 72$	$2n = 96$	$2n = 120$
A	Scattered	*D. linkii* *D. longifolia* *D. carphoides* *D. semiannularis* *D. occidentalis* 31%	*D. linkii* and var. *fulva* *D. longifolia* *D. clelandii* *D. geniculata* *D. richardsonii* 31%	*D. linkii* var. *fulva* *D. induta* *D. pallida* 19%	*D. induta* *D. pallida* 13%	*D. induta* 6%
B	2 Rows	*D. caespitosa* *D. setacea* *D. acerosa* aff. *D. alpicola* *D. auriculata* *D. duttoniana* *D. monticola* 50%	*D. caespitosa* *D. setacea* var. *breviseta* *D. eriantha* 21%	*D. caespitosa* *D. bipartita* *D. purpurascens* 21%	*D. procera* 7%	
C	Reduced	*D. laevis* *D. pilosa* and var. *paleacea* *D. nivicola* *D. nudiflora* *D. penicillata* *D. racemosa* *D.* sp. indescr. 70%	*D. laevis* *D. pilosa* and var. *paleacea* 20%	*D. laevis* 10%		

uniformly over the back of the lemma. Group B has the hairs arranged in two distinct horizontal rows or rows of tufts across the lemma, with the rest of the body of the lemma glabrous. Group C has one or both of the rows or tufts reduced or absent and the remainder of the back of the lemma glabrous. The most extreme example of group C is the new species shortly to be described by Mr. M. Gray (personal communication). This species has an almost glabrous lemma body and also has the callus ring of hairs reduced or absent. All other Australian species have the callus ring of hairs present (Fig. 3).

These groups correspond to Vickery's second, third, and fourth series, with the exception that *D. frigida* has been excluded.

Table 2 shows the relationships between these taxonomic groups and chromosome numbers of the species.

It is apparent that the species in group A have the greatest range of ploidy, $2n = 24$ to 120, and the fewest diploids, 31%. Group B has a more restricted ploidy range, $2n = 24$ to 96, and more diploids, 50%. Group C has a still more restricted ploidy range, $2n = 24$ to 72, and still more diploids, 70%. This suggests an evolutionary pattern originating with ancestral diploids having scattered uniformly distributed hairs and progressing in two directions: first, an increased specialization of hair arrangement at the diploid level by genic evolution, depleting the diploid representatives of the ancestral group and increasing the proportion of diploids in the more specialized forms, and secondly, a chromosomal evolution through polyploidy, the older groups of species extending further (24 to 120) than the more specialized groups (24 to 72).

(*c*) *Meiosis and Fertility in Species*

In all of the species examined meiosis was regular. Chiasma frequency was relatively high (1·8 to 2 chiasmata per bivalent) and univalents occurred very rarely. Multivalents have not been observed in any of the species, even though the chiasma frequency was sufficiently high to allow the formation of multivalents in recently formed polyploids. Pollen and seed fertility of all species was high (Appendix II).

These observations suggest that the polyploid forms are either alloploid in origin or are stabilized autoploids.

The predominantly inbreeding system prevailing in the genus would seem to favour autoploidy, but field hybrids occur and provide a starting point for alloploidy.

(*d*) *Field Hybrids*

A number of plants, collected in the vegetative phase, had aberrant chromosome numbers, aberrant meiosis, and reduced pollen fertility and seed setting.

Such sterile perennial hybrids which have little or no chance of propagation by seeds provide ideal starting material for alloploid forms, either by somatic chromosome doubling or by the production of unreduced gametes.

These natural hybrids have relatively high chiasma frequencies, compared with the artificial hybrids (cf. Table 8), suggesting that the parental plants were fairly closely related, at least genetically if not morphologically.

(*e*) *Atypical Plants*

In addition to these confirmed field hybrids a number of plants were collected which, on morphological characters, were clearly not typical of any species. These plants had intermediate characters and character combinations from several species. All were polyploid and had regular meiosis, high pollen fertility, and high seed fertility.

TABLE 3

MEIOSIS AND POLLEN FERTILITY IN NATURAL HYBRIDS IN DANTHONIA

Taxonomic Affinity	2*n*	No. of Cells	Metaphase Pairing Mean No. of				Chiasmata /Potential Bivalent	Pollen Fertility (%)
			IV	III	II	I		
D. caespitosa	36	23			11·6	12·9	0·96	28
D. monticola	36	25		0·1	11·1	13·7	0·75	9
D. richardsonii	48	22		0·8	16·8	12·0	0·86	16
D. caespitosa	60	25	1·2	3·2	17·0	11·6	1·26	13

Seed fertility: In all cases less than 1% of florets contained seeds.

These plants could be recent amphidiploids resulting from interspecific hybridization followed by chromosome doubling. This would account for both their morphological character combinations and their high fertility and regular meiosis.

TABLE 4

ATYPICAL PLANTS OF DANTHONIA*

Taxonomic Affinity	2*n*	Number of Plants	Locality
D. monticola	48	1	Ebor, N.S.W.
D. caespitosa–setacea	48	6	Forbes, Cowra, N.S.W.
D. caespitosa–eriantha	48	3	Coonabarabran, West Wyalong, Armidale, N.S.W.

* All these plants had regular meiotic behaviour and high pollen fertility (> 80%).

(*f*) *Artificial Hybrids*

In an effort to investigate further the hypothesis of alloploid speciation, and to throw light upon the relationships between the diploid and polyploid species, an extensive series of hybridizations between different species and different levels of ploidy was attempted.

The results of these crosses were expressed in four classes: viable, lethal, and inviable hybrids, and unsuccessful crosses.

Viable hybrids were those in which the hybrid seeds gave rise to F_1 plants which grew normally and flowered.

Lethal hybrids were those in which the hybrid seeds germinated, but the F_1 plants died before flowering. Some F_1 seeds produced chlorophyll-deficient seedlings which died as soon as their seed reserves were exhausted; in other cases small green plants were produced which persisted for some time before dying; in a few, the plants survived for a period of 12 to 18 months but they were never vigorous and did not flower.

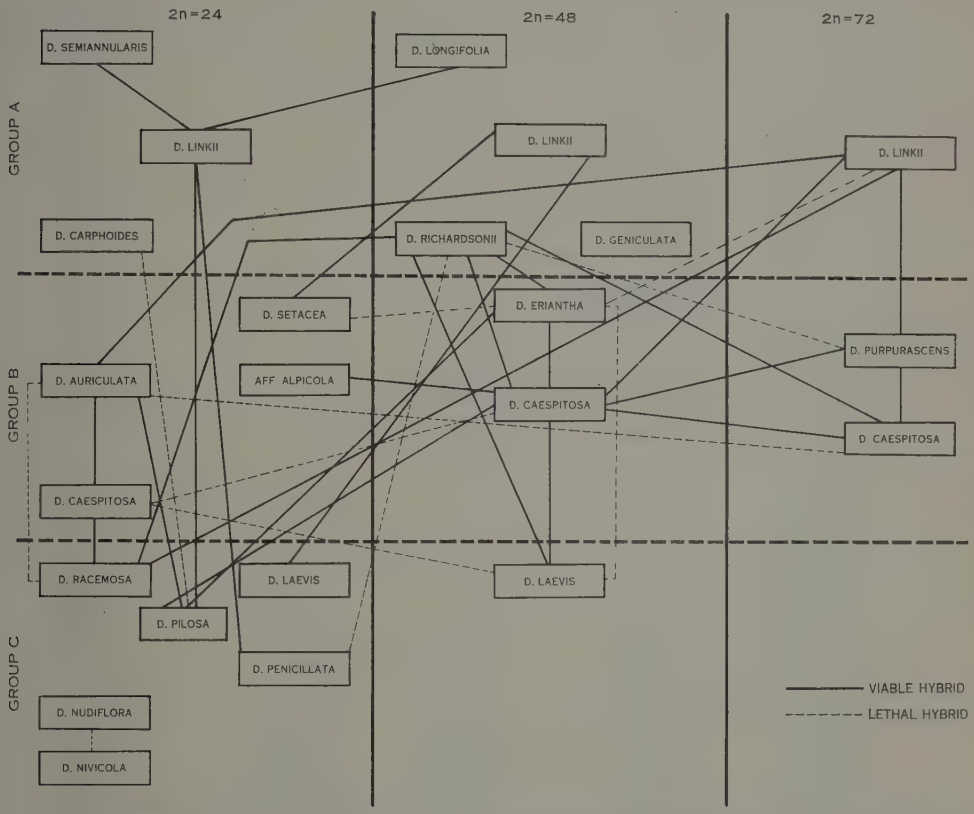

Fig. 1.—Success of hybridization (viable and lethal hybrids) of *Danthonia* species and the taxonomic grouping and chromosome numbers of the species involved.

Inviable hybrids were those in which a seed was formed, or there was evidence of fertilization and initial seed development, but no seed germination occurred. The seeds ranged from plump normal-looking seeds to shrivelled and abortive seeds which suggested endosperm failure.

Unsuccessful crosses were those in which there was no evidence of seed development. Because of the technical difficulties involved in making crosses in this genus we have less confidence in this class than in the others. Every care has been exercised to include only crosses where there was a reasonable certainty that the failure to hybridize was due to genetical and not to technical causes. Environmental factors undoubtedly had some influence on the outcome of any particular hybridizations, for when a series of florets was pollinated, either on one or on successive occasions,

there was some variation in the results. In these cases the result was recorded in the class of the most successful result achieved. In all crosses recorded as unsuccessful three or more florets were pollinated, usually on two or more separate occasions.

The results of these interspecific crosses are detailed in Appendixes III and IV, and in Figure 1 the successful (viable and lethal) hybrids are shown.

The lethal hybrid between the diploid and tetraploid forms of *D. caespitosa* is important in indicating that the genetic relationship between diploid and polyploid forms of a species is not necessarily close. This finding supports the view that the polyploidy is more likely to be alloploid than autoploid in origin.

(*g*) *Compatibility and Taxonomic Grouping*

The species compatibility in relation to taxonomic grouping is summarized in Table 5.

TABLE 5

CROSSABILITY IN RELATION TO TAXONOMIC GROUPING

Result of Crossing	I Within Taxonomic Group A×A B×B C×C	II Between Taxonomic Groups A×B B×C	III Between Taxonomic Groups A×C	Total
Viable hybrid	6	11	7	24
Lethal hybrid	4	7	3	14
Inviable hybrid	12	13	6	31
Unsuccessful	21	19	3	43
Total	43	50	19	112

Only 21·4% of the crosses resulted in viable F_1 plants or, if we include both viable and lethal, 33·9%. This high degree of genetic incompatibility between the species supports the view that speciation within the genus as measured by genetic isolation barriers is well advanced.

Grouping the various crosses as those between species which are morphologically similar (I), those less similar (II), and those most divergent (III) (Table 5), results in no positive correlation between taxonomic grouping and genetic compatibility. In fact a negative correlation is suggested. The more successful crosses (viable and lethal hybrids) occur more frequently between species in the taxonomically divergent groups than they do between species within the one taxonomic group; only 23·6% of the attempted crosses between morphologically similar species were successful (viable or lethal) whereas 52·6% of those between widely distinct species were successful (viable or lethal).

If we consider only the diploid×diploid crosses where the chromosome number effects can be eliminated, there is still no positive correlation with taxonomic grouping.

(*h*) *Compatibility and Chromosome Number*

The relationship which does emerge from this study of compatibility between species is a relationship with chromosome numbers. The higher the chromosome numbers of the parents, the more likely is the hybrid to be successful.

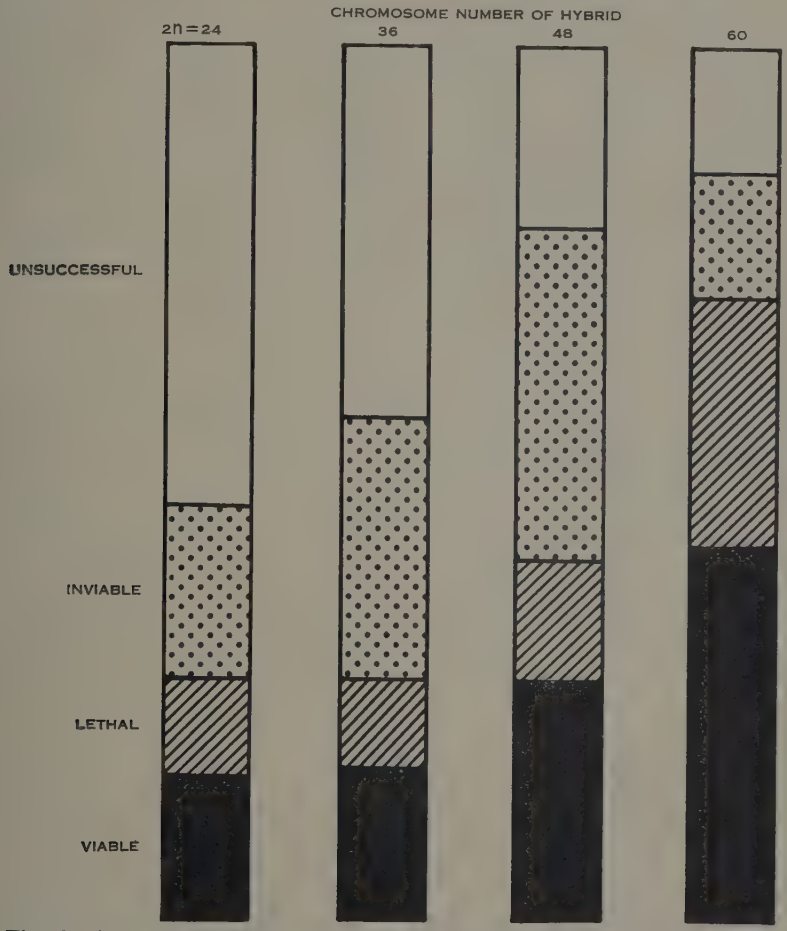

Fig. 2.—Relationship between success of crossing *Danthonia* species and chromosome number of the hybrid.

This greater genetic compatibility between polyploids reflects the greater buffering of multiple gene dosages and the increased tolerance of genetic unbalance in alloploids. It also supports the view that the higher polyploids have a range of diploid genotypes represented in their genetic make-up. Hence hybrids between polyploid species are more likely to have received similar genomes from each parent, and therefore have greater genetic compatibility than hybrids between diploid species.

(*i*) *Fertility of Viable Hybrids*

This view is further supported by the fertility status of the F_1 hybrids. The diploids are the most sterile, the triploids, tetraploids, and pentaploids more fertile.

TABLE 6

FERTILITY OF VIABLE HYBRIDS BETWEEN DANTHONIA SPECIES AND TAXONOMIC RELATIONSHIPS BETWEEN HYBRIDS AND PARENTS

Hybrid	2n	Taxonomic Group		Fertility (%)	
		Parents	Hybrid	Pollen	Seed Set
Diploids					
D. linkii 2n = 24 × *D. semiannularis* 2n = 24	24	A–A	A	0	0
D. caespitosa 2n = 24 × *D. auriculata* 2n = 24	24	B–B	B	1	0
D. auriculata 2n = 24 × *D. pilosa* 2n = 24	24	B–C	B	0	0
D. racemosa 2n = 24 × *D. caespitosa* 2n = 24	24	C–B	B	1	0
D. linkii 2n = 24 × *D. penicillata* 2n = 24	24	A–C	AB	0	0
D. linkii 2n = 24 × *D. pilosa* 2n = 24	24	A–C	B	0	0
			Mean	0·3	
Triploids					
D. linkii 2n = 24 × *D. longifolia* 2n = 48	36	A–A	A	0	0
D. caespitosa 2n = 48 × aff. *D. alpicola* 2n = 24	36	B–B	B	1	0
D. linkii 2n = 48 × *D. setacea* 2n = 24	36	A–B	A	17	0
D. caespitosa 2n = 48 × *D. pilosa* 2n = 24	36	B–C	B	2	0
D. pilosa 2n = 24 × *D. eriantha* 2n = 48	36	C–B	B	27	2
D. racemosa 2n = 24 × *D. richardsonii* 2n = 48*	36	C–A	B	3	0
D. racemosa 2n = 24 × *D. richardsonii* 2n = 48*	36	C–A	B	1	0
D. linkii 2n = 48 × *D. laevis* 2n = 24	36	A–C	AB	0	0
			Mean	6·4	

* Two hybrids with common *D. racemosa* parent but different *D. richardsonii* parents.

TABLE 6 (*Continued*)

Hybrid	2*n*	Taxonomic Group		Fertility (%)	
		Parents	Hybrid	Pollen	Seed Set
Tetraploids					
D. eriantha 2*n* = 48 × *D. caespitosa* 2*n* = 48	48	B–B	B	3	0
D. richardsonii 2*n* = 48 × *D. eriantha* 2*n* = 48	48	A–B	BA	4	0
D. caespitosa 2*n* = 48 × *D. richardsonii* 2*n* = 48	48	B–A	BA	16	0
D. auriculata 2*n* = 24 × *D. linkii* 2*n* = 72	48	B–A	A	10	0
D. laevis 2*n* = 48 × *D. caespitosa* 2*n* = 48	48	B–C	B	0	0
D. linkii 2*n* = 72 × *D. racemosa* 2*n* = 24	48	A–C	BA	11	3
D. racemosa 2*n* = 24 × *D. linkii* 2*n* = 72	48	C–A	BA	45	2
D. richardsonii 2*n* = 48 × *D. laevis* 2*n* = 48	48	A–C	B	5	0
				Mean 11·8	
Pentaploids					
D. caespitosa 2*n* = 48 × *D. caespitosa* 2*n* = 72	60	B–B	B	0	0
D. caespitosa 2*n* = 48 × *D. purpurascens* 2*n* = 72	60	B–B	B	11	0
D. purpurascens 2*n* = 72 × *D. caespitosa* 2*n* = 48	60	B–B	B	15	0
D. richardsonii 2*n* = 48 × *D. caespitosa* 2*n* = 72	60	A–B	BA	20	0
D. linkii 2*n* = 72 × *D. caespitosa* 2*n* = 48	60	A–B	BA	21	0
D. caespitosa 2*n* = 48 × *D. linkii* 2*n* = 72	60	B–A	A	7	0
				Mean 12·3	

The high sterility of the diploid hybrids has been maintained over two successive flowering periods and no seeds resulting from selfing have been obtained. This high level of sterility would probably preclude the functioning of these plants as parents even with a fully fertile partner. No back crosses were attempted but colchicine was applied to cuttings of a number of these hybrids and some tetraploid shoots were obtained. In these tetraploid shoots pollen fertility was restored to a level comparable with that of the parent species and seed setting after self-pollination was also frequent.

The higher fertility of the triploid, tetraploid, and pentaploid hybrids would permit these to function at least to a small extent as parents in further hybridization in the field. Even in the high chromosome number hybrids, seed setting after selfing was extremely low although occasional plump seeds were found when the plants were grown under field conditions for a number of seasons.

TABLE 7

FERTILITY OF A COLCHICINE-INDUCED TETRAPLOID OF A STERILE F_1 DIPLOID HYBRID BETWEEN D. LINKII ($2n = 24$) × D. PENICILLATA ($2n=24$)

	Pollen Fertility (%)	Seed Set (%)
Diploid hybrid $2n = 24$	0	0
Induced tetraploid $2n = 48$	94	71

(*j*) *Taxonomy of the Viable Hybrids*

The high degree of infertility of the F_1s precluded any attempt to study the inheritance of morphological characters in F_2 and later generations. Floret characters were examined in the F_1s and these throw some further light on the mode of species formation in this genus (see Appendix V).

Hybrids within the major taxonomic groups expressed, as would be expected, the morphology common to the group. Parents with scattered lemma hair arrangements produced F_1s with scattered hairs. Parents with two discrete rows of lemma hairs produced F_1s with two rows of hairs. However, it is notable that among this latter group the tetraploid *D. eriantha* × *D caespitosa* hybrid in addition to having two rows, also had some scattered hairs on the back of the lemma.

Hybrids between the groups were more interesting and some typical results are presented in Figure 3.

Hybrids between species with scattered hairs (group A) and those with two rows of hairs (group B) produced F_1s with two rows of hairs plus some scattered hairs. The number of scattered hairs varied between different crosses but showed no obvious relationship with ploidy of the parents or the F_1.

Hybrids between species of group B and species of group C (reduced rows of hairs) again tended to be intermediate in hair arrangement. The hairs were still present as two distinct rows but the density of the tufts was notably reduced.

Hybrids from species of group A (scattered hairs) with species of group C (reduced rows) in every case produced two rows of hairs, which is characteristic of group B. In some cases the lemma was glabrous between the rows as with the *D. linkii* × *D. pilosa* F_1 illustrated in Figure 3; in others, occasional scattered hairs were present.

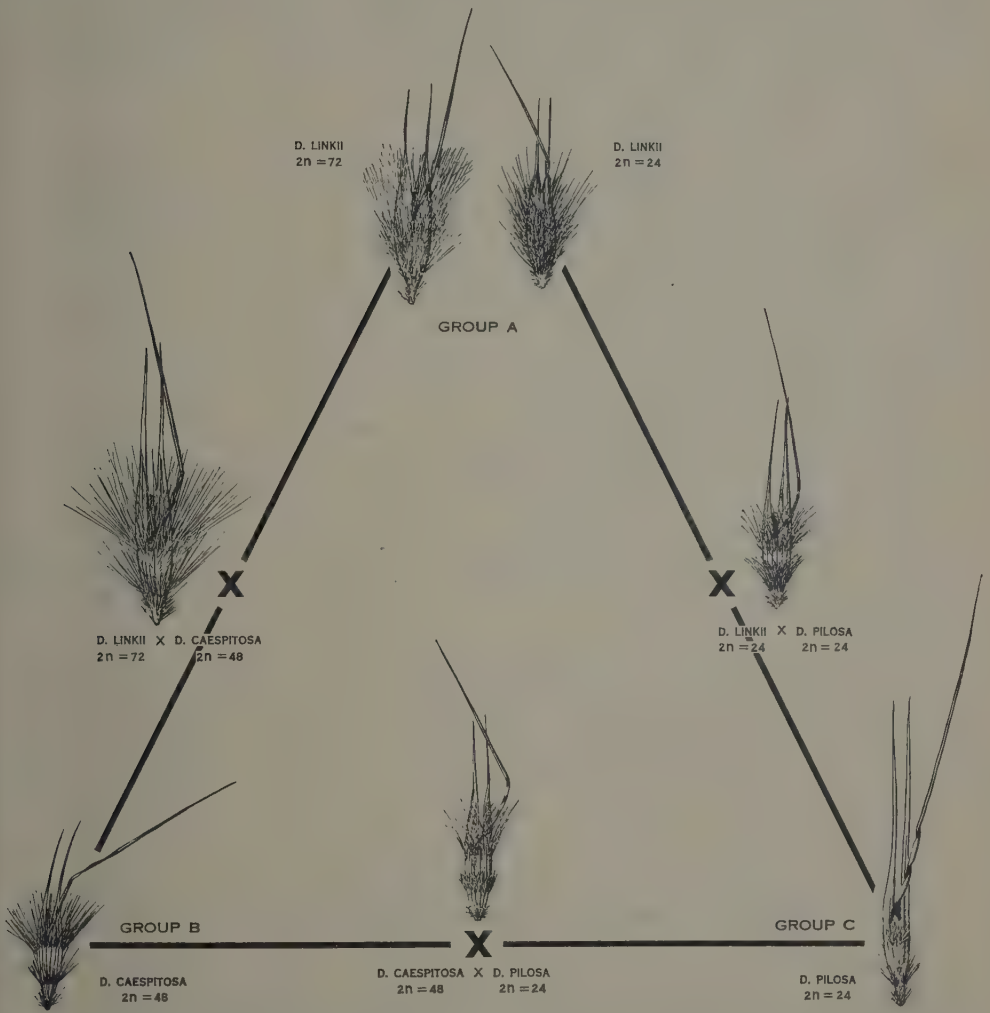

Fig. 3.—Typical examples of the floret morphology of F_1 hybrids between the three major taxonomic groups.

Other floral characters of the F_1s, such as shape and size of the floret, and length of lateral lobes and of the central awn tended to be intermediate between the parent species. The shape of the palea seems to be inherited independently from that of the lemma, for in a number of hybrids the palea shape resembled one parent whereas the lemma shape resembled the other parent.

The auricle which is characteristic of *D. auriculata* behaved as a recessive character. *D. auriculata* hybrids with *D. linkii* (group A), *D. caespitosa* (group B), and *D. pilosa* (group C) all lacked an auricle in the F_1s.

The expression of group B morphological characters in F_1s between species of group A × group C is notable in that it represents the potential synthesis of new species. Such F_1s are, of course, sterile, but polyploidy has been shown to restore fertility (Table 7) and such polyploids would have both the high fertility and the stability of genetic characters necessary for specific rank.

(*k*) *Meiosis in Viable Hybrids*

Meiotic pairing and chiasma frequency in all of the viable hybrids were very low, indicating very little residual chromosome homology between the different species. Multivalent associations in the diploid hybrids, quadrivalents in the triploids, and pentavalents in the tetraploids indicate the occurrence of translocations but no evidence of inversions was observed. The large number of univalents in all hybrids, together with the drastically reduced chiasma frequency, indicates a large amount of chromosomal or genic sterility.

The gross chromosomal aberrations in the diploid F_1s, as revealed by the multivalent associations, are insufficient to account for the observed pollen sterility. However, this sterility can be largely accounted for by the large number of univalents. Univalent formation can be either genic or chromosomal in origin. The restoration of fertility by chromosome doubling (Table 7) indicates that at least in this case the additional sterility is largely cryptic structural rather than genic in origin (Stebbins and Vaarama 1954).

In some of the polyploid F_1s there is a suggestion of some residual genome homology. For example, in the triploid F_1s of *D. linkii* × *D. setacea*, *D. pilosa* × *D. eriantha*, and *D. racemosa* × *D. richardsonii*, and in the tetraploid F_1s of *D. richardsonii* × *D. eriantha*, *D. caespitosa* × *D. richardsonii*, and *D. auriculata* × *D. linkii*, approximately 24 chromosomes paired, with the remaining chromosomes occurring as univalents.

The tetraploid hybrid resulting from crossing the diploid *D. racemosa* with the hexaploid *D. linkii* had a high degree of pairing, considerably in excess of that expected from homology of the single *D. racemosa* genome with one of the genomes of the hexaploid *D. linkii*. This high degree of pairing, and particularly the occurrence of quadrivalents and pentavalents, is unexpected, for both of the parent lines exhibit only bivalents at meiosis. This observation represents either the expression of gross structural rearrangements, or residual genomic homologies between the ancestors of the hexaploid *D. linkii*, the expression of which is suppressed at the normal meiosis of the species but revealed in the hybrid. Such a situation is analogous to the homoeologous pairing of *Triticum vulgare* which is revealed in this case by the removal of chromosome V (Riley, Chapman, and Kimber 1959).

The meiotic behaviour of these hybrids is singularly unsuccessful in showing any relationship with the taxonomic grouping of the parent species. The only relationship of this nature which can be seen is within the diploid hybrids. Here, multi-

TABLE 8

MEIOSIS AND TAXONOMIC RELATIONSHIPS OF VIABLE DANTHONIA HYBRIDS

Hybrid	No. of Cells	Metaphase Pairing Mean No. of					Chiasma Frequency		Taxonomy of Parents
		V	IV	III	II	I	/Potential Bivalent	/Observed Bivalent	
Diploids									
D. linkii $2n = 24 \times D.$ *semiannularis* $2n = 24$	17	—	—	—	9·28	5·43	1·02	1·32	A–A
D. caespitosa $2n = 24 \times D.$ *auriculata* $2n = 24$	16	—	—	—	2·18	18·50	0·23	1·00	B–B
D. auriculata $2n = 24 \times D.$ *pilosa* $2n = 24$	26	—	—	0·38	8·65	5·53	1·03	1·42	B–C
D. racemosa $2n = 24 \times D.$ *caespitosa* $2n = 24$	16	—	—	0·37	4·12	14·62	0·48	1·21	C–D
D. linkii $2n = 24 \times D.$ *penicillata* $2n = 24$	41	—	0·10	0·34	5·15	12·20	0·58	1·15	A–C
D. linkii $2n = 24 \times D.$ *pilosa* $2n = 24$	18	—	—	—	4·67	14·67	0·40	1·04	A–C
Triploids									
D. linkii $2n = 48 \times D.$ *setacea* $2n = 24$	12	—	0·75	1·50	7·50	13·83	0·92	1·42	A–B
D. caespitosa $2n = 48 \times D.$ *pilosa* $2n = 24$	40	—	—	1·35	6·52	18·90	0·55	1·09	B–C
D. pilosa $2n = 24 \times D.$ *eriantha* $2n = 48$	15	—	—	1·60	9·60	12·00	0·93	1·42	C–B
D. racemosa $2n = 24 \times D.$ *richardsonii* $2n = 48$*	25	—	0·38	2·40	9·40	8·88	1·06	1·41	C–A
D. racemosa $2n = 24 \times D.$ *richardsonii* $2n = 48$*	18	—	—	1·34	9·72	12·39	0·84	1·26	C–A
Tetraploids									
D. eriantha $2n = 48 \times D.$ *caespitosa* $2n = 48$	25	—	—	0·40	7·76	31·28	0·37	1·09	B–B
D. richardsonii $2n = 48 \times D.$ *eriantha* $2n = 48$	25	—	—	0·04	13·24	21·40	0·59	1·08	A–B
D. caespitosa $2n = 48 \times D.$ *richardsonii* $2n = 48$	25	—	0·08	0·80	9·44	26·40	0·61	1·36	B–A
D. auriculata $2n = 24 \times D.$ *linkii* $2n = 72$	24	—	—	0·75	11·25	23·25	0·64	1·38	B–A
D. linkii $2n = 72 \times D.$ *racemosa* $2n = 24$	26	0·08	0·73	2·04	16·42	5·73	1·18	1·30	A–C
D. racemosa $2n = 24 \times D.$ *linkii* $2n = 72$	20	—	0·50	1·90	17·00	6·30	1·17	1·31	C–A

* Two hybrids with common *D. racemosa* parent but different *D. richardsonii* parents.

valent associations, indicative of major structural rearrangements, do not occur in the hybrids between species of the same major taxonomic group, but they do occur in the hybrids between these groups. Also, with the exception of the *D. caespitosa* × *D. auriculata* hybrid, there is a general decrease of chiasma frequency and pairing as the morphology of the parent species becomes more divergent.

However, if any such relationship does exist at the diploid level it is completely absent in the triploid and tetraploid hybrids. In fact there is a strong suggestion of the reverse situation applying, i.e. chiasma frequency and pairing tend to increase as the parents become morphologically more diverse. This would indicate closer genetic relationship between the high polyploids and taxonomically unrelated diploids than between the members of a polyploid series within a species. Again this suggests alloploid origin of the high polyploids.

IV. Discussion

(*a*) *Origin of the Australian Species*

The evidence presented here suggests that the ancestral Australian *Danthonia* species had a low chromosome number, either $2n = 12$ or $2n = 24$, and had scattered hairs on the backs of the lemma. Evolution has utilized both genic and chromosomal changes, resulting in an increasingly specialized series of "diploid" species ($2n = 24$) and an extensive series of alloploid species.

De Wet (1960) after a study of African, North American, New Zealand, and some Australian species of *Danthonia* considered that the following features were characteristic of primitive species: poor development of lateral lobes of the lemma, scattered hair arrangement on the back of the lemma, non-localized distribution of chloroplasts in the leaf mesophyll, and the presence of dumb-bell-shaped siliceous cells and bicellular hairs on the leaf epidermis. No South African species exhibited all of these characters but the true diploids ($2n = 12$) *D. arundinacea*, *D. curva*, *D. disticha*, and *D. macowanii* all had some primitive characteristics. No evidence of any true diploid ($2n = 12$) Australian species was found in this investigation but the evidence presented is compatible with the postulate that the species of group A constitute the primitive group.

In the evolution of the Australian species there is evidence of both genic and chromosomal change (Table 8 and Appendix V). The occurrence of multivalent chromosomal associations at meiosis of the F_1 hybrids indicates the presence of translocated segments but there is no evidence for inversion. The failure of the gross structural rearrangements to account for the reduced fertility and the restoration of fertility by chromosome doubling (Table 7) indicates the presence of cryptic structural rearrangements.

The distribution of polyploids within the taxonomic groups, the occurrence of field hybrids and atypical polyploid plants, the results of artificial hybridization and the meiotic behaviour of the viable hybrids all support the view that the polyploid species are largely if not completely alloploid in origin. Further support is given by a comparison of the extent of variation in morphological characters occurring in diploid and polyploid members of the same species. If the chance hybridization of

two species, followed by chromosome doubling, results in a fertile, true-breeding form, then each separate event is likely to result in a polyploid differing somewhat from all of the others. Thus one would expect, on the basis of this mode of species formation, to have a wider range of variation exhibited by the polyploid members of a species than by the diploids. An examination of the variation in floral morphology, principally the arrangement of the lemma hairs, was made of 69 *D. caespitosa* plants whose chromosome number had been determined (Table 9). This examination revealed that 67% of the diploid plants had the typical *D. caespitosa* morphology of two rows of

TABLE 9

NUMBERS OF PLANTS WITH TYPICAL AND ATYPICAL FLORET MORPHOLOGY IN CHROMOSOME RACES D. CAESPITOSA AND D. LAEVIS

	Floret Morphology	
	Typical	Atypical
D. caespitosa		
$2n = 24$	6	3
$2n = 48$	16	38
$2n = 72$	2	4
D. laevis		
$2n = 24$	7	0
$2n = 48$	0	3
$2n = 72$	1	0

hairs on the back of the lemma and glabrous between, and only 33% with additional scattered hairs. However, of the tetraploids only 30% were typical and 70% atypical, and of the hexaploids 33% were typical and 67% atypical. The atypical polyploid forms often were atypical in lemma or palea shape in addition to having scattered hairs on the body of the lemma.

A similar situation applied with *D. laevis* where, although there was less material available for examination, all seven diploids were typical for that species in having the lower row of hairs absent, whereas the three tetraploids were all atypical in having some tufts of hairs present in the lower row. The single hexaploid examined was typical in having only the upper row of hairs.

These Australian species represent more extensive development of polyploid species than reported for the species of Africa or North America. Nine of the 29 species examined occur with more than one chromosome number and, as very few representatives of some species were examined, this number is not exhaustive. In addition, the variation in chromosome number is considerably greater than reported for the genus for other countries. De Wet (1954*b*) reports a range from $2n = 12$ to $2n = 72$ for South African species, which can be compared with the range from

$2n = 24$ to $2n = 120$ reported here for the Australian species. This increase of chromosomal variation illustrates at least one of the forms of genetic variation which the genus has utilized in colonizing new environments.

De Wet (1954*a*, 1954*b*) in studies of the genus *Danthonia* in relation to grass phylogeny pointed out that *Danthonia* constituted a diverse group of species on the basis of leaf anatomy. Four African species had the chloroplasts of the leaves confined to circular bands surrounding the vascular bundles (Panicoid chloroplast arrangement) and these differed from other species in the genus which had the chloroplasts uniformly distributed throughout the mesophyll of the leaves (Festucoid type). These four African species differed so obviously from the other *Danthonia* species that a new genus, *Asthenatherum* Nevski, was described to include them. The remaining species all had Festucoid chloroplast arrangements but varied in epidermal characteristics. Some had bicellular hairs and dumb-bell-shaped siliceous cells (Panicoid-type epidermis); others lacked bicellular hairs and had spherical siliceous cells (Festucoid); yet others had epidermis with a mixture of the two previous characters, i.e. both spherical and dumb-bell-shaped siliceous cells and bicellular hairs present or absent ("mixed" epidermis). The six Australian species which De Wet examined (1954*b*) all had Panicoid epidermis and Festucoid chloroplast arrangement which he considered ancestral for the genus. Examination of the 29 species reported in this paper confirmed the Panicoid type of epidermis and Festucoid type of chloroplasted arrangement for 23 of the species. The six exceptions were *D. procera*, *D. pallida*, *D. frigida*, *D. nivicola*, *D. nudiflora*, and *D.* sp. indescr. All had Festucoid-type chloroplast arrangements but varied in their epidermal characteristics.

In *D. pallida* the siliceous cells were reduced to very small plates and bicellular hairs were absent or rare.

D. procera and *D.* sp. indescr. had "mixed" epidermal characteristics, both dumb-bell-shaped and small spherical siliceous cells being present. Bicellular hairs were present.

D. frigida had large circular siliceous cells but lacked bicellular epidermal hairs.

D. nivicola and *D. nudiflora* both had bicellular hairs but had small spherical siliceous cells of the Festucoid type.

The limited amount of variation in leaf anatomy of the Australian *Danthonia* species, compared with the drastic rearrangements present in the world species, supports the view that the Australian species were isolated at an early stage. The mixed epidermal characters of *D. frigida* provide a further link with the alpine New Zealand species *D. cunninghamii* and *D. raoulii*, both of which have mixed epidermal characters as well as the same chromosome number (De Wet 1954*b*). The presence of small spherical siliceous cells in two other alpine species, *D. nivicola* and *D. nudiflora*, casts doubt on the suggested relationship of these species to the other Australian species of group C (Vickery 1956) and suggests a closer relationship with other New Zealand alpine forms such as *D. nuda*, which like *D. procera* and *D.* sp. indescr. has both dumb-bell and spherical siliceous cells plus bicellular hairs.

(*b*) *Taxonomy of the Australian Species*

The cytological and cytogenetic investigations reported here throw some light on some of the problems raised by Vickery in her taxonomic revision of the Australian species.

While chromosome size is known to be under genotypic control (Swanson 1957), the aberrant chromosome size of *D. bipartita* (Plate 1), together with its aberrant morphology and ecology, supports Vickery's opinion that this species is distinct from the other Australian species. Unfortunately it was not possible to get concurrent flowering of *D. bipartita* and other species so that its crossing relationships could be checked.

The different basic number for *D. frigida* ($x = 7$) confirms the lack of relationship of this species with other Australian species which all have a basic number of $x = 6$, and suggests a close relationship with alpine New Zealand species which have the same chromosome number ($2n = 42$).

The demonstration of polyploid series in a number of the Australian species and an alloploid mechanism of speciation explains the morphological variability and the tendency of species to merge into one another. The variability in *D. caespitosa* has been shown to be restricted largely to the polyploid forms (Table 9). The chromosome races of *D. linkii* and *D. linkii* var. *fulva* form an inter-grading polyploid series, all 8 diploid forms examined being *D. linkii*, all 9 hexaploids being *D. linkii* var. *fulva*, and 6 of the 11 tetraploids being *D. linkii*, the remaining 5 *D. linkii* var. *fulva*.

Thus some of the suggestions made by Vickery as to the relationships between the species seem very plausible in the light of this further information, others are not so plausible. For example, the development of her fourth group of species, which have reduced lemma hairs, from *D. caespitosa* through *D. pilosa* to *D. racemosa* and *D. penicillata*, is consistent with a diploid evolutionary series and is in general supported by crossing relationships. *D. pilosa* and *D. racemosa* both show a relatively high degree of cross compatibility with other species, *D. racemosa* and *D. pilosa* producing viable hybrids with diploid and tetraploid *D. caespitosa* respectively. However, the relationship of *D. nudiflora* and *D. nivicola* with the other species in this group is not supported by either crossing relationships or leaf anatomy. Other suggestions made by Vickery, such as *D. monticola* being related to *D. geniculata* "perhaps after hybridization with *D. purpurascens*", cannot be correct, for while *D. monticola* undoubtedly hybridizes with some species in the New England area (field hybrids with $2n = 36$ were collected in addition to diploids with $2n = 24$), neither *D. geniculata*, which is a tetraploid ($2n = 48$), nor *D. purpurascens*, which is a hexaploid ($2n = 72$), could have been directly involved in the evolution of the diploid *D. monticola*.

(*c*) *Plant Breeding*

As *Danthonia* species constitute a major component of unimproved pastures, thought has been given from time to time to the production of improved strains. While no attempt to produce improved strains for agricultural use was made, this work indicates clearly the problems which would be encountered if conventional

plant breeding methods were employed in such endeavours. Species crosses are difficult to achieve, particularly between species of low chromosome number, and the fertility of F_1 hybrids is far too low to permit the production of the large F_2 populations necessary for selection. However, the problems of sterility could be overcome by selection of desirable F_1 plants and the production of fully fertile alloploids by chromosome doubling. Should agronomic considerations warrant the initiation of plant breeding work in *Danthonia*, a method such as this would have to be followed unless crossing was to be restricted to intraspecific lines.

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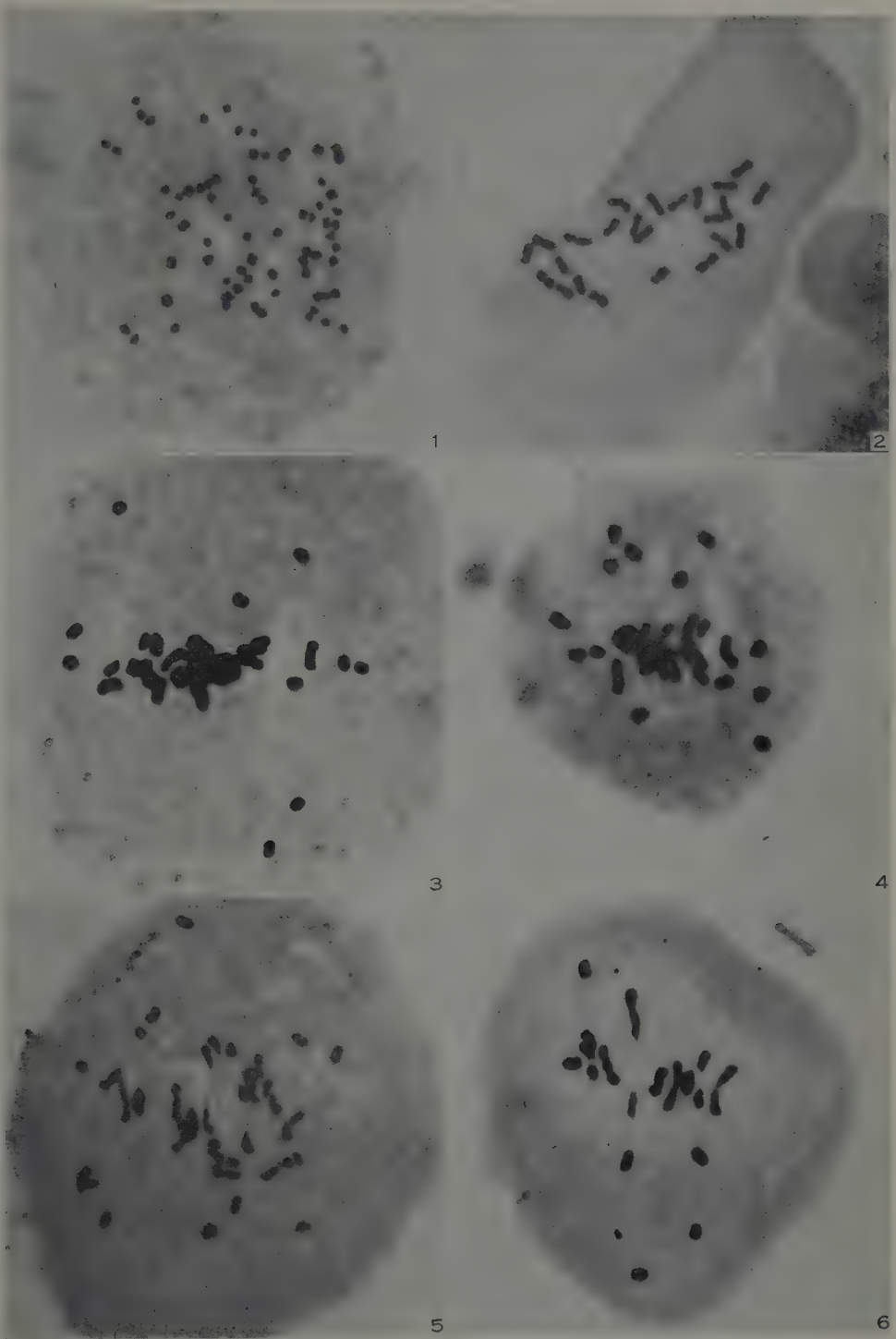
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Explanation of Plate 1

Somatic and meiotic chromosomes of some *Danthonia* species and hybrids. All × 1250.

Fig. 1.—*D. bipartita* $2n = 72$. Somatic metaphase from root-tip squash.

Fig. 2.—*D.* sp. indescr. $2n = 24$. Somatic metaphase from root-tip squash.

Fig. 3.—First metaphase configuration of field hybrid aff. *D. caespitosa* $2n = 60$, showing univalents.

Fig. 4.—First metaphase configuration of field hybrid aff. *D. monticola* $2n = 36$, showing 12 bivalents and 12 univalents.

Fig. 5.—First metaphase configuration of artificial hybrid between *D. auriculata* $2n = 24 \times D.$ *linkii* var. *fulva* $2n = 72$, showing 1 trivalent, 10 bivalents, and 25 univalents.

Fig. 6.—First metaphase configuration of artificial hybrid between *D. linkii* $2n = 24 \times D.$ *penicillata* $2n = 24$, showing 2 trivalents, 6 bivalents, and 6 univalents.

Appendix I

Chromosome numbers and distribution of Danthonia species

Species	2*n*	Locality*
D. acerosa J. Vickery	24	Darkan, Bunbury, W.A.
aff. *D. alpicola* J. Vickery	24	Junction Shaft, Tolbar, Snowy Mts., N.S.W.
D. auriculata J. M. Black	24	Canberra, A.C.T.
D. bipartita F. Muell.	72	W. of Oodnadatta, S.A.; Alice Springs, Erldunda, Kulgera, N.T.; Mundiwindi, W.A.
D. caespitosa Gaud.	24	Canberra, A.C.T.; Gilgandra, Cowra, Trangie, N.S.W.; Port Augusta, Terowie, S.A.
	48	Canberra, A.C.T.; Coonabarabran, Forbes, West Wyalong, Cowra, Wagga, Barraba, N.S.W.; Nyah, Vic.; Binnum, Peterborough, Robe, Verdun, Mt. Lofty, Naracoorte, S.A.; Doodlakine, Clackline, W.A.
	72	Canberra, A.C.T.; Cowra, N.S.W.; Ambleside, Ungarra, S.A.
D. carphoides F. Muell.	24	Canberra, A.C.T.; Blayney, N.S.W.
D. clelandii J. Vickery	48	Bridgewater, S.A.
D. duttoniana Cashmore	24	Marsden, West Wyalong, Cowra, N.S.W.; Binnum, S.A.
D. eriantha Lindl.	48	Dubbo, Boorowa, Adaminaby, Armidale, Coonabarabran, Marsden, N.S.W.; Naracoorte, S.A.
D. frigida J. Vickery	42	Mt. Kosciusko, 6000–7000 ft, N.S.W.
D. geniculata J. M. Black	48	Tooperang, S.A.
D. induta J. Vickery	72	Mt. Kosciusko, N.S.W.
	96	Armidale, N.S.W.
	120	The Grampians, Vic.
D. laevis J. Vickery	24	Canberra, A.C.T.; Armidale, Kiandra, Yarrangobilly, Mt. Kosciusko, Blayney, N.S.W.
	48	Springton, Verdun, S.A.
	72	Wandsworth, N.S.W.
D. linkii Kunth	24	Gilgandra, Tamworth, Guyra, Glen Innes, Armidale, N.S.W.
	48	Armidale, Tamworth, Murrurundi, Singleton, Camden, N.S.W.
D. linkii var. *fulva* J. Vickery	48	Camden, Adaminaby, N.S.W.
	72	Camden, Temora, West Wyalong, N.S.W.; Canberra, A.C.T.
D. longifolia R. Br.	24	Armidale, N.S.W.
	48	Bruthen, Vic.
D. monticola J. Vickery	24	Moredun, N.S.W.
D. nivicola J. Vickery	24	Mt. Kosciusko, 5700–7000 ft, N.S.W.
	48	Mt. Kosciusko, 5700–7000 ft, N.S.W.
D. nudiflora P. F. Morris	24	Mt. Kosciusko, 5700–6800 ft, Bendemeer, Kiandra, N.S.W.
D. occidentalis J. Vickery	24	Bunbury, W.A.
D. pallida R. Br.	72	Canberra, A.C.T.; Bathurst, N.S.W.
	96	Coonabarabran, N.S.W.

APPENDIX I (*Continued*)

Species	2*n*	Locality*
D. penicillata (Labill.) Beauv.	24	Orange, Guthega, Mt. Kosciusko, and Snowy Mts., N.S.W.
D. pilosa R. Br.	24	Talbingo, Wandsworth, Mt. Kosciusko, N.S.W.; Canberra, A.C.T.
D. pilosa var. *paleacea* J. Vickery	24	Waterfall Gully, S.A.
	48	Fergerson, W.A.
D. procera J. Vickery	96	Ballarat, Vic.
D. purpurascens J. Vickery	72	Armidale, Putty, Mittagong, Picton, Boorowa, N.S.W.
D. racemosa R. Br.	24	Blayney, Coonabarabran, Armidale, Guyra, Wandsworth, Singleton, Talbingo, Bathurst, Wee Jasper, Moredun, N.S.W.; Kybybolite, S.A.
D. racemosa var. *obtusata* F. Muell.	24	Armidale, Bendemeer, N.S.W.
D. richardsonii Cashmore	48	Boorowa, Cowra, Armidale, Tamworth, Murrurundi, Bathurst, N.S.W.
D. semiannularis (Labill.) R. Br.	24	Lower Gellibrandt, Vic.
D. setacea R. Br.	24	Cowra, Dubbo, Alectown, Forbes, Singleton, West Wyalong, Henty, N.S.W.; Collie, W.A.
D. setacea var. *breviseta* J. Vickery	48	Lake Wagin, W.A.
D. sp. indescr. (M. Gray, personal communication)	24	Mt. Kosciusko, N.S.W.

* Collecting localities are given here as the nearest town. Exact localities are lodged with the herbarium specimen in the Herbarium of the Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T.

Appendix II

Meiosis and pollen fertility in some Danthonia species

Species	2n	No. of Cells	Metaphase Pairing Mean No. of		Chiasmata Frequency /Potential Bivalent	Pollen Fertility (%)
			II	I		
D. auriculata	24	16	12·0		1·98	92
D. caespitosa	24	21	12·0		1·83	98
D. carphoides	24	30	11·7	0·5	1·94	86
D. linkii	24	51	12·0		1·94	96
D. penicillata	24	24	12·0		2·00	98
D. pilosa	24	20	12·0		1·96	96
D. racemosa	24	25	12·0		1·95	97
D. racemosa var. *obtusata*	24	24	11·9	0·2	1·92	91
D. setacea	24	25	12·0		1·87	94
D. frigida	42	22	20·9	0·1	1·60	92
D. caespitosa	48	22	24·0		2·01	95
D. eriantha	48	19	24·0		2·01	93
D. geniculata	48	21	24·0		1·96	94
D. linkii	48	22	24·0		1·98	96
D. linkii var. *fulva*	48	28	24·0		1·96	94
D. richardsonii	48	25	24·0		1·96	94
D. bipartita	72	18	36·0		1·94	94
D. caespitosa	72	28	36·0		1·90	97
D. laevis	72	16	36·0		1·89	93
D. linkii var. *fulva*	72	28	36·0		1·85	98
D. purpurascens	72	26	36·0		1·89	97
D. induta	96	14	48·0		1·84	96
D. procera	96	8	48·0		1·88	92
D. induta	120	5	60·0		1·83	98

In addition to these species the following species all had pollen fertility in excess of 90%:

$2n = 24$	$2n = 48$	$2n = 72$	$2n = 96$
aff. *D. alpicola*	*D. clelandii*	*D. induta*	*D. pallida*
D. duttoniana	*D. laevis*	*D. pallida*	
D. laevis	*D. longifolia*		
D. longifolia	*D. nivicola*		
D. monticola			
D. nivicola			
D. nudiflora			
D. semiannularis			

Appendix III

RESULTS OF DANTHONIA SPECIES CROSSES

Viable	Lethal	Inviable	Unsuccessful
Diploid × diploid			
D. auriculata × *D. pilosa*	*D. auriculata* × *D. racemosa*	*D. auriculata* × *D. caespitosa*	*D. auriculata* × *D. carphoides*
D. caespitosa × *D. auriculata*	*D. nudiflora* × *D. nivicola*	*D. nivicola* × *D. nudiflora*	*D. auriculata* × *D. semiannularis*
D. linkii × *D. semiannularis*	*D. pilosa* × *D. carphoides*	*D. nudiflora* × *D. racemosa*	*D. caespitosa* × *D. duttoniana*
D. linkii × *D. penicillata*	*D. pilosa* × *D. auriculata*	*D. pilosa* × *D. caespitosa*	*D. caespitosa* × *D. setacea*
D. linkii × *D. pilosa*		*D. pilosa* × *D. linkii*	*D. duttoniana* × *D. caespitosa*
D. racemosa × *D. caespitosa*		*D. racemosa* × *D. auriculata*	*D. duttoniana* × *D. setacea*
		D. racemosa × *D. penicillata*	*D. laevis* × *D. pilosa*
			D. nivicola × *D. caespitosa*
			D. nivicola × *D. duttoniana*
			D. nivicola × *D. pilosa*
			D. nivicola × *D. setacea*
			D. nudiflora × *D. duttoniana*
			D. pilosa × *D.* aff. *alpicola*
			D. pilosa × *D. setacea*
			D. racemosa × *D. linkii*
			D. racemosa × *D. nivicola*
			D. racemosa × *D. nudiflora*
			D. setacea × *D. caespitosa*
			D. setacea × *D. carphoides*
Diploid × tetraploid			
D. linkii × *D. longifolia*	*D. caespitosa* × *D. laevis*	*D. auriculata* × *D. richardsonii*	*D. caespitosa* × *D. caespitosa*
D. pilosa × *D. eriantha*	*D. setacea* × *D. eriantha*	*D. duttoniana* × *D. caespitosa*	*D. caespitosa* × *D. laevis*
D. racemosa × *D. richardsonii*		*D. laevis* × *D. linkii*	*D. duttoniana* × *D. geniculata*
		D. nudiflora × *D. laevis*	*D. duttoniana* × *D. laevis*
		D. pilosa × *D. richardsonii*	*D. nudiflora* × *D. caespitosa*
		D. racemosa × *D. linkii*	*D. nudiflora* × *D. richardsonii*
			D. pilosa × *D. laevis*
			D. racemosa × *D. caespitosa*
			D. racemosa × *D. geniculata*

APPENDIX III (*Continued*)

Viable	Lethal	Inviable	Unsuccessful
Diploid × hexaploid			
D. auriculata × *D. linkii*	*D. auriculata* × *D. caespitosa*		*D. pilosa* × *D. caespitosa*
D. racemosa × *D. linkii*			*D. racemosa* × *D. caespitosa*
Tetraploid × diploid			
D. caespitosa × aff. *D. alpicola*	*D. caespitosa* × *D. caespitosa*	*D. caespitosa* × *D. racemosa*	*D. caespitosa* × *D. auriculata*
D. caespitosa × *D. pilosa*	*D. richardsonii* × *D. penicillata*	*D. eriantha* × *D. pilosa*	*D. caespitosa* × *D. duttoniana*
D. linkii × *D. laevis*		*D. eriantha* × *D. racemosa*	*D. caespitosa* × *D. nivicola*
D. linkii × *D. setacea*		*D. eriantha* × *D. setacea*	*D. caespitosa* × *D. setacea*
		D. laevis × *D. racemosa*	*D. eriantha* × *D. auriculata*
		D. richardsonii × *D. racemosa*	*D. geniculata* × *D. caespitosa*
			D. laevis × *D. nudiflora*
			D. longifolia × *D. semiannularis*
Tetraploid × tetraploid			
D. caespitosa × *D. richardsonii*	*D. eriantha* × *D. richardsonii*	*D. caespitosa* × *D. geniculata*	*D. caespitosa* × *D. eriantha*
D. eriantha × *D. caespitosa*	*D. laevis* × *D. eriantha*	*D. caespitosa* × *D. laevis*	*D. longifolia* × *D. richardsonii*
D. laevis × *D. caespitosa*	*D. laevis* × *D. geniculata*	*D. eriantha* × *D. laevis*	*D. richardsonii* × *D. linkii*
D. richardsonii × *D. eriantha*		*D. geniculata* × *D. caespitosa*	
D. richardsonii × *D. laevis*		*D. laevis* × *D. nivicola*	
Tetraploid × hexaploid			
D. caespitosa × *D. linkii*	*D. eriantha* × *D. linkii*		
D. richardsonii × *D. caespitosa*	*D. richardsonii* × *D. purpurascens*		

APPENDIX III (*Continued*)

Viable	Lethal	Inviable	Unsuccessful
Hexaploid × diploid			
D. linkii × *D. racemosa*		*D. linkii* × *D. auriculata* *D. linkii* × *D. caespitosa* *D. linkii* × *D. carphoides* *D. linkii* × *D. laevis* *D. linkii* × *D. linkii* *D. purpurascens* × *D. racemosa*	*D. caespitosa* × aff. *D. alpicola*
Hexaploid × tetraploid			
D. purpurascens × *D. caespitosa*		*D. linkii* × *D. linkii*	*D. caespitosa* × *D. richardsonii*

In addition to these hybrids the following viable hybrids made by Miss E. L. Robertson at the Waite Agricultural Research Institute were included in this study:

D. caespitosa $2n = 48 \times D.$ *caespitosa* $2n = 72$
D. caespitosa $2n = 48 \times D.$ *purpurascens* $2n = 72$
D. caespitosa $2n = 48 \times D.$ *linkii* $2n = 72$
D. linkii $2n = 72 \times D.$ *caespitosa* $2n = 48$
D. purpurascens $2n = 72 \times D.$ *caespitosa* $2n = 48$
D. caespitosa $2n = 72 \times D.$ *purpurascens* $2n = 72$
D. purpurascens $2n = 72 \times D.$ *linkii* $2n = 72$
D. purpurascens $2n = 72 \times D.$ *purpurascens* $2n = 72$

Appendix IV

RECIPROCAL DIFFERENCES IN CROSSES BETWEEN DANTHONIA SPECIES

Cross	Result of Cross	Result of Reciprocal Cross
Diploid × diploid		
D. auriculata × *D. pilosa*	Viable	Lethal
D. linkii × *D. pilosa*	Viable	Inviable
D. auriculata × *D. racemosa*	Lethal	Inviable
D. nudiflora × *D. nivicola*	Lethal	Inviable
D. nudiflora × *D. racemosa*	Inviable	Unsuccessful
Diploid × tetraploid		
D. racemosa × *D. richardsonii*	Viable	Inviable
D. setacea × *D. eriantha*	Lethal	Inviable
D. caespitosa × *D. caespitosa*	Lethal	Unsuccessful
D. caespitosa × *D. laevis*	Lethal	Unsuccessful
D. linkii × *D. laevis*	Inviable	Viable
D. duttoniana × *D. caespitosa*	Inviable	Unsuccessful
D. nudiflora × *D. laevis*	Inviable	Unsuccessful
D. racemosa × *D. caespitosa*	Unsuccessful	Inviable
Diploid × hexaploid		
D. auriculata × *D. linkii*	Viable	Inviable
Tetraploid × tetraploid		
D. eriantha × *D. richardsonii*	Viable	Lethal
D. laevis × *D. caespitosa*	Viable	Inviable
D. eriantha × *D. caespitosa*	Viable	Unsuccessful

Appendix V

INHERITANCE OF FLORET CHARACTERS IN DANTHONIA INTERSPECIFIC F_1 HYBRIDS

Hybrid	F_1 Genome	Lemma		Palea Shape
		Hair Distribution	Shape	
D. linkii 2*n* = 24 × *D. semiannularis* 2*n* = 24	AA	Scattered	aff. *D. linkii*	aff. *D. semiannularis*
D. linkii 2*n* = 24 × *D. longifolia* 2*n* = 48	AAA	Scattered	aff. *D. linkii*	
D. caespitosa 2*n* = 24 × *D. auriculata* 2*n* = 24	BB	2 Rows	aff. *D. caespitosa* (no auricle)	aff. *D. auriculata*
D. caespitosa 2*n* = 48 × aff. *D. alpicola* 2*n* = 24	BBB	2 Rows	aff. *D. caespitosa*	aff. *D. alpicola*
D. eriantha 2*n* = 48 × *D. caespitosa* 2*n* = 48	BBBB	2 Rows + occasional scattered hairs	aff. *D. caespitosa*	Intermediate
D. caespitosa 2*n* = 48 × *D. purpurascens* 2*n* = 72	BBBBB	2 Rows + scattered hairs	Intermediate	aff. *D. caespitosa*
D. linkii 2*n* = 48 × *D. setacea* 2*n* = 24	AAB	2 Rows + occasional scattered hairs	aff. *D. linkii*	aff. *D. linkii*
D. richardsonii 2*n* = 48 × *D. eriantha* 2*n* = 48	AABB	,, ,,	aff. *D. richardsonii*	aff. *D. eriantha*
D. caespitosa 2*n* = 48 × *D. richardsonii* 2*n* = 48	AABB	,, ,,	aff. *D. caespitosa*	aff. *D. richardsonii*
D. richardsonii 2*n* = 48 × *D. caespitosa* 2*n* = 72	AABBB	,, ,,	aff. *D. caespitosa*	Intermediate
D. auriculata 2*n* = 24 × *D. linkii* 2*n* = 72	AAAB	,, ,,	aff. *D. linkii* (no auricle)	Intermediate
D. linkii 2*n* = 72 × *D. caespitosa* 2*n* = 48	AAABB	2 Rows + scattered hairs	aff. *D. linkii*	Intermediate
D. linkii 2*n* = 24 × *D. penicillata* 2*n* = 24	AC	2 Rows	aff. *D. penicillata*	aff. *D. penicillata*
D. linkii 2*n* = 24 × *D. pilosa* 2*n* = 24	AC	2 Rows + occasional scattered hairs	aff. *D. pilosa*	aff. *D. linkii*
D. racemosa 2*n* = 24 × *D. richardsonii* 2*n* = 48	AAC	,, ,,	Intermediate	Intermediate
D. linkii 2*n* = 48 × *D. laevis* 2*n* = 24	AAC	,, ,,	aff. *D. linkii*	aff. *D. laevis*
D. richardsonii 2*n* = 48 × *D. laevis* 2*n* = 48	AACC	,, ,,	aff. *D. laevis*	aff. *D. laevis*
D. racemosa 2*n* = 24 × *D. linkii* 2*n* = 72	AAAC	,, ,,	aff. *D. linkii*	aff. *D. linkii*
D. racemosa 2*n* = 24 × *D. caespitosa* 2*n* = 24	BC	2 Rows, upper row reduced	aff. *D. caespitosa*	aff. *D. racemosa*
D. auriculata 2*n* = 24 × *D. pilosa* 2*n* = 24	BC	2 Rows	aff. *D. pilosa* (no auricle)	aff. *D. auriculata*
D. caespitosa 2*n* = 48 × *D. pilosa* 2*n* = 24	BBC	Reduced rows	aff. *D. pilosa*	aff. *D. pilosa*
D. pilosa 2*n* = 24 × *D. eriantha* 2*n* = 48	BBC	2 Rows	Intermediate	aff. *D. pilosa*

COMPETITION BETWEEN *EUCALYPTUS MELLIODORA* AND *E. ROSSII* AT VARYING LEVELS OF EXCHANGEABLE CALCIUM

By C. W. E. Moore*

[*Manuscript received August* 5, 1960]

Summary

E. rossii and *E. melliodora* were grown in pot culture as competing species at varying levels of exchangeable calcium.

Both height and yield of *E. melliodora* increased with increasing exchangeable calcium.

Regressions of log ratio *E. rossii*/*E. melliodora* for both height and yield on exchangeable calcium were highly significant ($P<0\cdot001$) and there was no evidence of departure from linearity.

The data indicate that plant growth is influenced by the degree of calcium saturation of the soil rather than by the absolute amount of exchangeable calcium.

The implications of the results in relation to the natural distribution of the two species are discussed.

I. Introduction

Investigations of the nutrient status of the soils of some natural plant communities on the Southern Tablelands of New South Wales have shown that in general the soils supporting a dry sclerophyll forest with dominant *E. rossii* Baker and Smith are grossly deficient in exchangeable calcium (Moore 1959*a*). The level of exchangeable calcium in the soil may be one factor affecting the delimitation of the dry sclerophyll forest and the neighbouring savannah woodland with dominant *E. melliodora* A. Cunn.

Moore (1959*b*) has also shown that the community which develops on a particular site will depend not only on the environmental conditions at that site, but also on the presence or absence of competing species. In studies of specific environmental factors on the development of a species the effect of interspecific competition must be examined, especially when one of the objectives of the investigation is to gain an understanding of the natural distribution of species and communities in the field.

To test the hypothesis that the level of exchangeable calcium in the soil is a factor affecting the natural distribution of *E. melliodora* and *E. rossii*, the two species were grown in competition in pot culture at different levels of exchangeable calcium. Results which might be expected to apply in the field would not be obtained in the absence of interspecific competition, and hence the effect of calcium on the individual species in pure stand was not determined.

* Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T.

II. Material and Methods

Soil was obtained from the *E. rossii* community at site 1 (Moore 1959*a*) as the level of exchangeable calcium was lower in this soil than in any others examined. In the soil sample obtained for the studies on nutritional status, no exchangeable calcium could be detected chemically.

Previous studies have given no indication as to whether plant growth is affected by the absolute amount of exchangeable calcium in the soil or by the degree of calcium saturation. In the original samples (Moore 1959*a*) the total exchange capacity of the soil obtained from the *E. rossii* community was 17·7 m-equiv. %,

Table 1

EXCHANGEABLE CALCIUM AND PERCENTAGE CALCIUM ON THE EXCHANGE COMPLEX IN FIVE PAIRS OF SOILS FROM E. ROSSII AND E. MELLIODORA COMMUNITIES

Site	*E. rossii* Soil		*E. melliodora* Soil	
	Exchangeable Ca (m-equiv. %)	Ca on Exchange Complex (%)	Exchangeable Ca (m-equiv. %)	Ca on Exchange Complex (%)
1	0·0	0·0	2·3	24
2	0·1	0·9	4·3	43
3	0·4	3·3	6·8	50
4	0·1	0·6	4·0	40
5	4·4	25·3	10·4	60

while that for the neighbouring *E. melliodora* community was 9·7 m-equiv. %. The experiment reported here was therefore divided into two sections. In section *A* the soil was mixed with fine quartz sand to reduce its total exchange capacity to approximately 10%, while in section *B* the soil was used without dilution.

The soils were placed in enamel pots of $4\frac{1}{2}$ in. (11·5 cm) diameter, containing 1490 g air-dry sand–soil mixture per pot (section *A*) or 1200 g air-dry soil per pot (section *B*). Moisture equivalents were determined for the sand–soil mixture and for the undiluted soil.

The level of exchangeable calcium was varied by addition of calcium hydroxide. The required quantity was thoroughly mixed with the soil in each pot. The calcium hydroxide was added on July 21, 1958, 4 months before sowing the seed. On August 4, 1958, water was added to all pots to bring the moisture level to the moisture equivalent. The moisture content of the soil was thereafter maintained at or near the moisture equivalent by regular weighing and watering. This allowed a reasonable time for the added calcium to reach equilibrium with the soil and enter the exchange complex.

The treatments were based on the data obtained in the studies of the nutrient status of soils (Moore 1959*a*). Table 1 shows the exchangeable calcium and the

TABLE 2

HEIGHT AND YIELD OF TOPS (GEOMETRIC MEANS) OF E. ROSSII AND E. MELLIODORA AND RATIO OF MEANS

	Exchangeable Ca (m-equiv. %)	Height (cm)			Yield of Tops (g)		
		E. rossii	*E. melliodora*	*E. rossii*/ *E. melliodora*	*E. rossii*	*E. melliodora*	*E. rossii*/ *E. melliodora*
Section *A*	0·30	15·7	9·9	1·58	2·18	0·48	4·52
	0·38	17·0	10·9	1·55	2·34	0·62	3·74
	0·46	17·2	10·9	1·58	2·51	0·61	4·08
	0·54	17·6	10·3	1·71	2·47	0·54	3·54
	0·61	16·7	9·7	1·73	2·46	0·51	4·85
	0·69	18·2	10·5	1·74	2·74	0·57	4·78
	0·77	17·5	12·5	1·40	2·15	0·76	2·83
	0·85	16·5	12·6	1·31	2·17	0·79	2·76
	0·93	16·3	12·5	1·31	2·25	0·77	2·92
	1·01	18·9	11·1	1·70	2·65	0·65	4·07
	1·08	15·6	12·4	1·34	2·48	0·73	3·40
	1·24	15·0	12·0	1·26	2·12	0·70	3·01
	1·48	17·7	14·2	1·24	2·52	0·98	2·57
	1·63	17·0	13·3	1·28	2·55	0·97	2·62
	1·87	17·1	13·9	1·23	2·47	0·92	2·69
	2·26	18·4	13·1	1·40	2·69	0·83	3·24
	2·80	17·5	12·6	1·39	2·52	0·83	3·03
	4·69	18·8	19·0	0·99	3·29	1·45	2·28
	5·90	16·7	23·4	0·71	2·41	2·02	1·19
Section *B*	0·89	17·5	10·7	1·63	2·75	0·67	4·10
	1·42	19·0	11·0	1·72	3·16	0·71	4·48
	1·94	17·9	11·8	1·52	2·95	0·67	4·42
	2·98	17·4	13·3	1·31	2·64	0·93	2·84
	5·07	17·7	16·1	1·10	3·05	1·18	2·59
	8·86	19·4	18·6	1·10	3·67	1·58	2·32

percentage of calcium on the exchange complex in the five pairs of soils obtained from the *E. rossii* and *E. melliodora* communities.

It is clear that both species may occur naturally on soils with a wide range of exchangeable calcium, but in view of the low values for the *E. rossii* soils, apart from the exceptional case at site 5 (Moore 1959*a*, p. 346), it seemed likely that one might expect a change in dominance at a relatively low level of exchangeable calcium.

Seed of the two species was sown on the surface of the soil in each pot on November 18, 1958, and the resulting seedlings were subsequently thinned to retain one plant of each species per pot. The thinning was done in a number of stages to ensure that the final plants were healthy, undamaged individuals approximately uniform in size throughout the experiment. The thinning was completed on February 20, 1959.

Sixteen pots were prepared for each calcium treatment, one of which was reserved for obtaining soil samples for chemical analysis, leaving 15 replications. Subsequent losses reduced the number of replications in section *A* to 9 and in section *B* to 12, each section being arranged in a separate randomized block design.

The plants were harvested on November 15, 1959. Height and oven-dry weight of tops were recorded (drying at 80°C).

Exchangeable calcium, sodium, potassium, magnesium, and hydrogen and total exchange capacity were determined at the time of sowing the seed on samples of the soil obtained from the special pots set aside for this purpose, the methods being the same as those reported previously (Moore 1959*a*).

III. Results

The values for exchangeable calcium as determined chemically agreed well with calculated values, indicating that the added calcium had entered the exchange complex. Treatments had no effect on the exchangeable sodium, potassium, magnesium, or total exchange capacity, but with increasing calcium there was naturally a decrease in exchangeable hydrogen. Calculated values for exchangeable calcium have been accepted as being more reliable than chemical determinations as differences of the order of 0·1 m-equiv. % cannot be chemically detected.

The results are summarized in Table 2, which shows the geometric means for tree heights and top weights of each species, together with the ratios of the means.

Increasing levels of exchangeable calcium had little or no effect on height or yield of *E. rossii*, but there was a marked effect on both height and yield of *E. melliodora*. The ratio *E. rossii*/*E. melliodora* for both height and yield decreased with increasing exchangeable calcium.

The regressions of the log ratios *E. rossii*/*E. melliodora* for height and yield on exchangeable calcium in both the sand–soil mixture and the undiluted soil were highly significant ($P<0{\cdot}001$) with no evidence of departure from linearity.

IV. Discussion

On the Southern Tablelands of New South Wales, dry sclerophyll forest with dominant *E. rossii* and savannah woodland with dominant *E. melliodora* frequently occur as neighbouring communities with sharp boundaries or narrow ecotones. There is evidence that the distribution of these communities is limited by one or more edaphic factors, and that the level of exchangeable calcium in the soil may be one of the factors involved (Moore 1959*a*).

It is clear, however, that the tolerances of *E. melliodora* and *E. rossii* overlap in respect of at least some of the factors involved (Moore 1959*b*). That this applies to exchangeable calcium is clear from an examination of Table 1 above.

Studies of the serpentine and highly acid soils of California have revealed that plant and community distribution are affected by both edaphic and biotic factors. Kruckeberg (1954) pointed out that serpentine endemics are confined to serpentine soils in part by their tolerance of the unusual edaphic conditions and in part by their inability to compete with the non-serpentine flora. McMillan (1956) suggests that "the plant community is the result of the over-lapping of different tolerance ranges of the component individuals for environmental conditions presented by a particular habitat".

It is likely that at the time of migration of *Eucalyptus* into the region seed of both species was available. Under these conditions the effect of interspecific competition is to favour one or other species on different soils, and we now have, in general, discrete communities which can be related to certain soil conditions. At the present time seed of both species is certainly available for colonization of disturbed areas, especially near the boundaries of the two communities, but there is no evidence of recent invasion of one community by another.

The results indicate that when *E. rossii* and *E. melliodora* are grown under conditions of competition, increasing levels of exchangeable calcium favour the development of *E. melliodora*, and it seems likely that at the higher levels of calcium this latter species would become the dominant in a natural community. On the basis of tree heights it would appear that *E. melliodora* would gain a clear advantage over *E. rossii* when the exchangeable calcium in the soil exceeds 4 m-equiv. % in a soil of total exchange capacity 9·48 m-equiv. % (42·2% calcium on the exchange complex). However, for the undiluted soil of exchange capacity 19·93 m-equiv. %, the exchangeable calcium must be as high as 9 m-equiv. % (47·1% calcium on the exchange complex) before *E. melliodora* can be expected to become dominant.

Data for weight of tops gave similar results, except that it appears that higher levels of calcium would be required to give full advantage to *E. melliodora* at the age at which the trees were harvested.

The factor involved appears to be the percentage of calcium on the exchange complex rather than the absolute amount of exchangeable calcium in the soil. It is of interest to note that Vlamis (1949) concluded that the limitation to growth on the serpentine soils of California is associated with the degree of calcium saturation of the soil.

The soil used for the work reported here was obtained from a typical stand of *E. rossii*. By increasing the level of exchangeable calcium, the soil could readily be converted to one which under natural conditions would probably develop a woodland community with *E. melliodora* as the dominant species. The mean value of 43·4% of calcium on the exchange complex for the *E. melliodora* communities (Table 1) agrees well with the data obtained in this work—42·2% for the sand–soil mixture and 49·1% for the undiluted soil.

V. Acknowledgments

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SOME MORPHOLOGICAL, ANATOMICAL, AND PHYSIOLOGICAL CHANGES IN THE PEAR FRUIT (*PYRUS COMMUNIS* VAR. WILLIAMS BON CHRÉTIEN) DURING DEVELOPMENT AND FOLLOWING HARVEST

By Joan M. Bain*

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Summary

Morphological, anatomical, and physiological changes occurring in the developing fruit of *Pyrus communis* var. Williams Bon Chrétien were studied at frequent intervals, from blossom until after commercial maturity, in three successive seasons.

Morphological changes were shown by increase in measurements of volume, long and short axis, and the width of the cortex (flesh), the morphology of the fruit being interpreted by the receptacular theory. Anatomical changes were given by the duration and distribution of cell division, differentiation of tissues, cell size, and cell number. Physiological changes were expressed as changes in fresh weight, dry weight, and moisture content for the whole fruit, and separately for the flesh, peel, and core in the second and third seasons. Total and reducing sugars, starch, titratable acidity, and total and protein nitrogen were estimated per gram of dried flesh at each sampling. Respiration rates for whole fruit were measured by the Pettenkofer method. Physiological changes could not be expressed on a per cell basis because of the complex structure of pear tissue.

Data presented on the basis of the number of days from blossom showed two distinct stages in fruit growth. Stage I, the first 42–56 days of development, corresponded to the main cell division period and was characterized by more rapid morphological but slower physiological changes (except for increase in protein nitrogen) than Stage II, the remainder of the time on the tree. Comparable trends were found in the three seasons, but drought reduced growth rate in the first season.

Some ripening changes were followed on removal from the tree and after periods of cold storage at 0°C.

I. Introduction

Data from samples of fruit collected at Bathurst, N.S.W., in three consecutive seasons have been used to correlate morphological, anatomical, and physiological changes occurring in the pear fruit var. Williams Bon Chrétien (also known as Bartlett) during its four months' development. Some post-harvest changes were also followed as the fruit ripened on removal from the tree and after varying periods of cold storage. Very marked changes in skin colour and fruit texture associated with a sharp rise in respiration during a short ripening period make the Williams pear very suitable material for the study of post-harvest changes.

A background to the anatomical structure and physiological behaviour of the pear fruit is found in the publications of McAlpine (1911); Murneek (1923); Crist and Batjer (1931); Mitchell (1950); Sterling (1954); Smith (1935); Martin (1937); Tindale, Trout, and Huelin (1938); Kidd *et al.* (1940); Ulrich and Thaler (1957);

* C.S.I.R.O. Division of Food Preservation, Ryde, N.S.W.

and Hulme (1958). Considerable information is available on the anatomy of the pear and on some aspects of its physiology during short periods, or throughout its entire development on the tree (especially for Williams). Nevertheless, a complete developmental study of the fruit has not been available up to now.

In the present work, the morphology of the fruit was interpreted according to the receptacular theory, with the cortex equivalent to the flesh, and with the pith, separated from it by a ring of 10 main vascular bundles, surrounding the five carpels. The core is defined as the five carpels + pith.

II. Material and Methods

(*a*) *Source of Material and Sampling*

Fruits used during the investigation were obtained from the New South Wales Department of Agriculture Experiment Farm at Bathurst, N.S.W. One 60-year-old tree was used as the experimental tree during the three seasons but fruit from a similar adjacent tree was also used in the early part (up to 54 days) of the third season because of lighter blossoming.

Samples were taken at intervals from full blossom until well after commercial maturity. Full blossom was estimated to be at October 8, 1956, October 4, 1957, and October 17, 1958, respectively for each successive season, and commercial picking was on February 18, 1957, February 5, 1958, and February 10, 1959—133, 124, and 116 days respectively from full blossom. The final experimental picks were taken on March 11, 1957, February 24, 1958, and March 3, 1959, after 154, 143, and 137 days respectively. Full blossom in the third season was late because of cool conditions, and commercial picking in the first season was delayed owing to previous drought conditions. Large numbers of fruits per sample were taken early in the season to ensure sufficient material for analysis. Later in the season the sample usually consisted of 40 fruits: 20 were used for the anatomical sample and 5 for the moisture determinations; these 25 fruits, together with another 5, were dried and sampled for the chemical analyses; the remaining 10 fruits were used in 2 batches of 5 each for the respiration measurements.

Fruits were picked at random from the tree, this sampling method being considered satisfactory enough to show seasonal trends in the developing fruit. Griggs and Iwakiri (1956) compared the growth rates of tagged Bartlett pears left on the tree with those of a sample picked at random, and concluded that small inconsistencies in growth rate in the randomized sample were of little practical importance when growth curves were used to show the relative effects of experimental treatments.

(*b*) *Observations at Each Sampling*

(i) *Morphological Changes.*—Morphological changes were measured at each sampling in each of the 20 fruits of the anatomical sample in the following way: (*a*) length of long axis from base of stalk to the calyx, (*b*) diameter of the fruit at its widest part, (*c*) width of the cortex, and the radius of the carpels plus the pith in the same position as (*b*). Fruit volume was measured by displacement.

(ii) *Anatomical Changes.*—Anatomical investigations were carried out at each sampling on material fixed in acetic-alcohol (1 : 3) and stored in 70% alcohol. Sections were hand-cut and mounted, usually unstained, in glycerine jelly. Observation was confined to the epidermis, hypodermis, cortex, and pith; the distribution of these tissues in the ovary is given in Plate 1, Figure 1.

(1) *Anatomy.*—Frequent observation, especially in the early stages of growth, established the position and duration of cell division and traced the differentiation of the tissues.

(2) *Cell Size.*—Cell size determinations were carried out on the parenchyma cells of the mid cortex, following methods previously used for apple fruits (Bain and Robertson 1951). A plug of tissue removed radially from the cortex in the equatorial region of each of 20 fruits was sectioned longitudinally. The outlines of four cells in the centre of each of four fields of a microprojector were traced from each of 20 sections, making a total of 320 cells per sample. These cells were then reduced in number to 80 by using the ranking method of McIntyre (1952). Cell size was expressed as cell volume (Bain and Robertson 1951) and in terms of the cell surface (Robertson and Turner 1951).

(3) *Cell Number.*—Cell number was calculated by dividing the mean parenchyma cell volume into the tissue volume (Smith 1938). Tissue volume was calculated from the fruit weight using a value of 1·1 for the specific gravity of the cells. The percentage air space in the fruit was calculated from the tissue volume.

(iii) *Physiological Changes on the Tree.*—Physiological data were expressed on a dry-weight and fresh-weight basis, "per fruit", "per cell", and "per unit of protein nitrogen". Analyses of carbohydrates, nitrogen, and acidity were done on finely-ground dried flesh plus peel in the 1956–57 season and on dried flesh without peel in the two following seasons; at least 30 fruits were dried to obtain the material for analysis at each pick. These values were converted to a fresh-weight basis from a knowledge of the moisture content of the material; then to a per fruit basis, calculated from the total fruit weight on the assumption that the whole fruit was like the flesh. This method gives average trends and ignores any variation between tissues, such as shown for Bosc pears by Martin (1936), or any gradient within these tissues. Respiration rates were determined on intact fruit.

The following determinations were carried out at each pick.

(1) *Fresh Weight.*—Average fresh weight of 20 stalked fruit was found in the anatomical sample. The weights of the peel, flesh, and core were calculated after approximately 8 wk in the second and third seasons using data from the moisture content sample. The stalk was included with the core.

(2) *Moisture Content and Total Solids.*—Thin slices of tissue were dried at 70°C in aluminium cans in an air draught oven for approximately 12 hr and then in a vacuum oven for 15 hr. The percentage moisture content was found for the flesh plus the peel in the 1956–57 season, the absence of the core over-estimating this value. The moisture contents in the peel, flesh, and core were determined separately in the 1957–58 and 1958–59 seasons and combined to give that for the whole fruit.

(3) *Carbohydrate Content.*—Total and reducing sugars were estimated according to Somogyi (1952). Non-reducing sugars were calculated by difference. Starch content was determined by a method after Nielsen (1943).

(4) *Nitrogen Content.*—Total nitrogen was estimated in 0·5 g of dried material by a modified micro-Kjeldahl method, the distillation being carried out in a modified Parnas-Wagner apparatus (McKenzie and Wallace 1954).

Protein nitrogen was estimated in 1·0 g of dried material following extraction with 75% alcohol (Turner 1949). Soluble nitrogen was recorded as their difference.

(5) *Titratable Acidity.*—Determinations of titratable acidity were made according to the methods of analysis of the Association of Official Agricultural Chemists (1955).

(6) *Respiration Rate.*—Respiration rate of whole fruit was measured by the Pettenkofer method on duplicate samples held at 20°C for several days following each sampling.

(7) *Estimation of Maturity.*—Maturity changes were followed by observing the colour changes of the peel and by using a Magness-Taylor pressure tester ($\frac{5}{16}$ in. diameter plunger) to estimate firmness of the flesh.

(iv) *Post-harvest Physiological Changes*

(1) *On Removal from Tree.*—Respiration rate was measured, as above, in fruit ripening after each sampling in the later part of the three seasons. Measurements were made until breakdown occurred.

(2) *Following Storage at 0°C.*—Commercially mature fruits were stored at 0°C in the second and third seasons and removals made after 20, 67, and 97 days in 1957–58, and after 22, 61, and 85 days in 1958–59. Ripening changes at 20°C were then followed at close intervals in groups of 10 fruit, a separate group being taken for each sampling within the removal. In the 1957–58 crop the 10 fruit were taken at random from the removal unit; in the 1958–59 seasons, groups of 10 fruit with an average weight close to that at commercial maturity were allocated prior to storage. Changes in colour, pressure, and respiration rate of the fruit, and the carbohydrate, nitrogen, and acidity level per gram dry weight of flesh, and its percentage moisture content, were followed in both seasons. The percentage weight loss during ripening was recorded for the fruit at each sampling in the 1958–59 season.

III. Results and Discussion

Morphological, anatomical, and physiological changes occurring in the developing fruit followed the same general trends in the three seasons, but as expected, the rate and magnitude of these changes varied from season to season with crop size and climatic conditions (Fig. 1). Severe drought during the greater part of the first season considerably reduced growth rate in the already heavy crop fruit, therefore data collected for the more favourable second and third seasons can be taken to be more typical of an average season. Growth rates in these two seasons were comparable even though the 1958–59 crop was light; lower average maximum monthly temperatures in the third season were possibly responsible for this.

Growth rate was highest in the 1958–59 season when it was expressed as time from blossom, but not when it was reckoned on a calendar basis (Fig. 2). Agreement between seasons was closer on a calendar basis in the maturing fruit, but the 11-day lag in blossoming in 1958 was evident in the early part of the season. These pears thus differ from developing Valencia orange fruits, which were found to have a shorter cell division period after a late blossoming, with the result that successive growth curves agreed when expressed on a calendar basis (Bain 1958). The pear

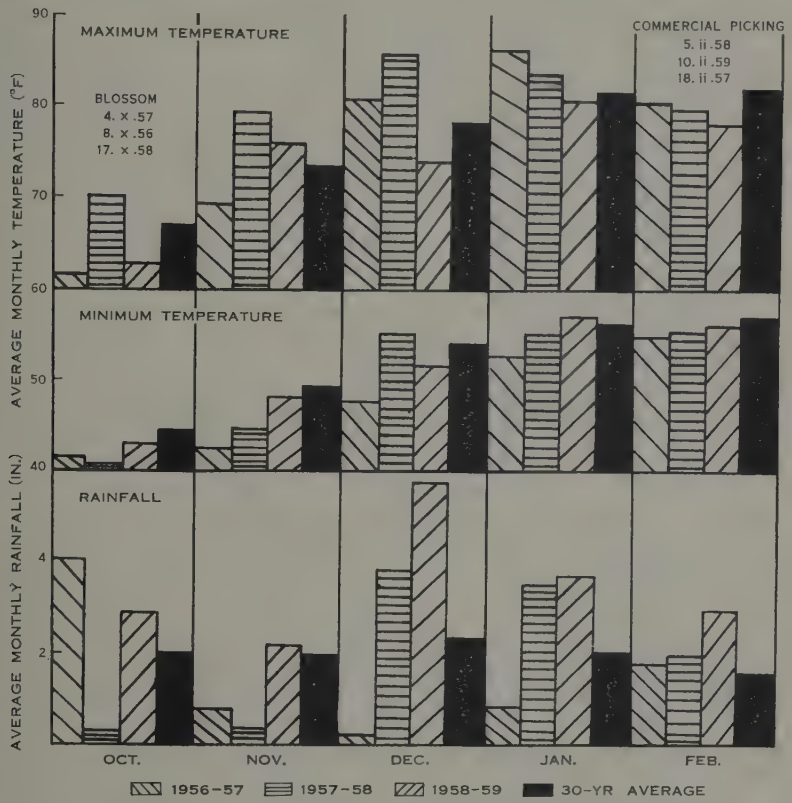

Fig. 1.—Climatic data for the Bathurst Experiment Farm (altitude 2341 ft, 33°26′, 149°33′) throughout the three growing seasons and the 30-yr monthly average (Bureau of Meteorology 1956).

data have therefore been presented on a "time from blossom" scale. Data for the three seasons have been given in the tables and, where practicable, in the diagrams. Anatomical data have been given in detail for the 1957–58 seasons.

Correlation of the anatomical and physiological data for the three seasons showed that fruit development could be divided conveniently into two stages. Stage I, the first 42–56 days from blossom, corresponded to the main cell division period and was characterized by a slow rate of physiological change. Stage II, the remainder of the period on the tree, was characterized by cell enlargement and

rapid physiological change. Morphological changes occurred at a faster rate in Stage I than in Stage II.

Attempts to express physiological changes per cell, as for the developing apple fruit (Robertson and Turner 1951; Pearson and Robertson 1953), were unsatisfactory because of the more complex structure of pear tissue, which contains groups of sclereids. The method of calculating cell number assumed that the average size of the parenchyma cells in the flesh is typical of the greater part of the fruit tissue after cell division has ceased. In the developing pear, however, the parenchyma cells represent only a portion of the flesh cells. Since these cells enlarge considerably,

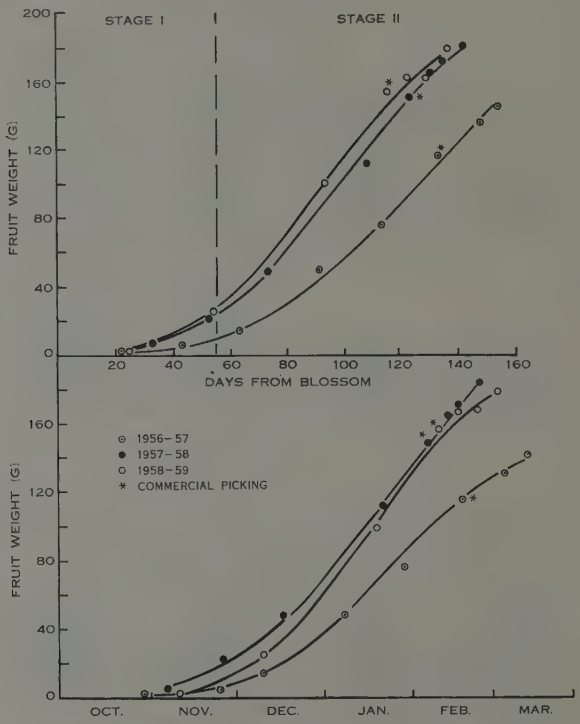

Fig. 2.—Changes in fruit weight plotted on a calendar and on a time-from-blossom basis.

unlike the sclereids, the average cell size for the tissue is over-estimated (Plate 1, Fig. 2), and an apparent decrease in cell number at each sampling is obtained. As parenchyma cells and sclereids were of comparable size at the end of the cell division period (Plate 2, Fig. 1), the cell number calculated at the end of Stage I was used for the rest of the season. Physiological changes per cell based on total cell number could not, however, be considered satisfactory when a high proportion of the cells were sclereids. The proportion of parenchyma in the tissue estimated from the area of the sclereid groups in a known field was approximately 65% of the total cell number at the end of Stage I. Physiological changes expressed per parenchyma cell, although still very approximate, would perhaps be more reliable except for changes in total dry weight, but data per cell have not been presented, except in Figure 8.

TABLE 1

MORPHOLOGICAL CHANGES OCCURRING DURING STAGES I AND II IN FRUIT DEVELOPMENT, 1956–57 SEASON*

Days from Blossom	Long Axis (mm)	Short Axis (mm)	Width of Cortex (mm)	Cortex as % of Radius	Radius of Core (mm)	Long Axis / Short Axis	Fruit Volume (c.c.)	Fruit Volume / (Long Axis)³	Fruit Volume / (Short Axis)³	Colour
Stage I										
0	3·3	3·1	0·7	43·8	0·9	1·1	0·1	3·89	4·61	
7	8·3	3·9	0·9	45·0	1·1	2·1	—	—	—	
14	12·5	4·6	1·4	60·9	0·9	2·7	—	—	—	
21	18·0	7·8	2·3	59·0	1·6	2·3	0·9	0·15	1·78	
43	33·0	20·2	6·9	68·3	3·2	1·6	4·2	0·12	0·51	
63	42·3	28·0	9·3	66·4	4·7	1·5	16·2	0·21	0·74	
Stage II										
91	58·2	42·0	13·6	64·8	7·4	1·4	49·0	0·25	0·66	
113	67·4	49·5	15·4	62·1	9·4	1·4	75·2	0·25	0·62	
133†	72·5	54·7	17·2	62·8	10·2	1·3	114·5	0·30	0·70	Light green
148	74·2	58·3	20·8	71·2	8·4	1·3	135·5	0·33	0·68	Light green
154	81·1	60·1	19·1	63·4	11·0	1·3	143·0	0·27	0·66	Green-yellow

* Data from the other two seasons did not differ appreciably.

† Commercial picking.

(a) *Stage I—The Cell Division Period*

(i) *Morphological Observations.*—Fruit volume and weight showed a linear relationship during this period, with specific gravity at approximately 1·0. Fruit shape altered considerably throughout and its irregularity made estimation of morphological changes difficult (Plate 3). Measurement of the long axis and short axis (including width of cortex and radius of core in equatorial position) gave some

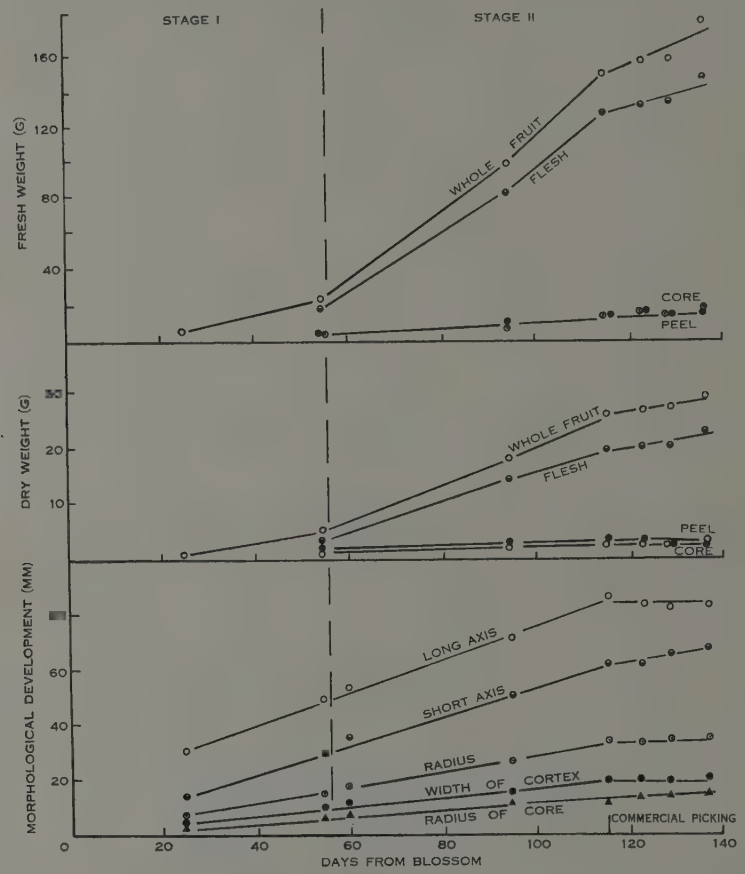

Fig. 3.—Changes in fresh weight, dry weight, and morphology during the 1958–59 season.

indication of these changes, but the proportion of the tissues in the fruit were not known (Table 1, Fig. 3). The cortex was 43·8% of the cross section of the ovary in 1956 and 66·4%, 53·0%, and 60·2% of the fruit radius at the end of Stage I in the three seasons respectively. Some change in shape was estimated by the ratio long axis/short axis, which was 1·1 at blossom, 2·3 at 21 days, and 1·5 at the end of Stage I in 1956, and also by using the constants fruit volume/(long axis)^3 and fruit volume/(short axis)^3 as given in Table 1 (Lilleland 1933).

(ii) *Anatomical Observations*

(1) *Structure of Ovary Wall at Blossom* (*Plate* 1, *Fig.* 1).—The exterior was slightly hairy and covered by a thin cuticle; the wall was approximately 40 cells wide. The outer tissues of the wall are shown in Plate 2, Figure 2. Radial cell division was frequent in the dense epidermal cells and occasional stomates occurred in this layer. The hypodermis was a single layer of cells larger but less dense than those of the epidermis and the cells were enlarged at the base of the hairs. Cell division was mostly radial, but tangential division was also seen. The cortex was approximately 30 rows wide, with the outer 7–10 rows distinguished from the rest of the thin-walled isodiametric cells by their smaller, roughly rectangular cells with dense contents. Isolated patches of cell division were found in this tissue. The pith, surrounding the carpels and separated from the cortex by a ring of 10 vascular bundles, was approximately 10 cells wide; these cells showed less frequent cell division and were smaller than the cortical cells and elongated tangentially.

The vascular system of pear fruit was interpreted by McAlpine (1911). The bundles entering from the stalk form a ring at the approximate junction of the cortex and pith. Five of these, each located behind a carpel, branch on the inner side to give a large bundle close to each carpel and the vascular network of the cortex, the other five bundles alternating with the carpels branch to form the carpellary vascular system to the inside and the cortical network outside.

(2) *Anatomical Changes.*—The detailed anatomical development in the above tissues is set out in Table 2.

Increase in fruit size was by increase in cell number in the first few weeks. Cell division, observed in patches in the ovary wall tissue, became very active in the outer cortex, cortex, and pith approximately 7 to 14 days from blossom. The main cell division period lasted approximately 28 days, during which time the hypodermis increased to 3, 4, or 6 layers, the outer cortex to 15, and the cortex to approximately 100 rows. A diagrammatic representation of the duration and intensity of cell division is given in Figure 4. Division was continuous in the epidermis, hypodermis, and outer cortex to keep pace with increasing fruit volume by the end of Stage I.

At blossom, the volume of the parenchyma cells of the cortex was approximately $0{\cdot}15 \times 10^{-4}$ cu.mm. Average cell volume increased slightly during the next 7 days but decreased again as the cell division period began; it increased to $0{\cdot}6 \times 10^{-4}$ cu.mm. by the end of Stage I in the first season and to $2{\cdot}0 \times 10^{-4}$ cu.mm. in the second and third seasons. Cell number increased from 5–10 million to approximately 100 million. Increase in cell size and cell number are given in Figure 5; the effect of drought was already evident by the end of Stage I in the first season.

Sclereids, responsible for the grittiness of pear tissue, began to form approximately 14 days from blossom when the walls of isolated cells, which appeared larger in the mass of dividing cortical and pith cells, became lignified (Plate 4, Fig. 1). Groups of sclereids formed in these tissues within the next 14 days, reaching up to 20 in cross section in the inner cortex/outer pith region. Rate of sclereid formation decreased in these tissues after 28 days from blossom, but additional sclereids were still developing at the margins of clusters at the end of Stage I (Plate 5, Fig. 2).

TABLE 2

ANATOMICAL CHANGES OCCURRING DURING DEVELOPMENT OF THE PEAR FRUIT (TRANSVERSE EQUATORIAL SECTION)

Days from Blossom	Epidermis	Hypodermis	Outer Cortex	Cortex	Pith	Occurrence of Cell Division
Stage I						
0 4.x.57	Cells radially elongated, contents dense (Pl. 2, Fig. 2). Covered by cuticle, slightly hairy, and interrupted by occasional stomates	Single layer—cells larger, less dense than epidermis (Pl. 2, Fig. 2). 2 or 3 enlarged cells at base of hairs	7–10 rows—cells dense, rounded or rectangular (Pl. 2, Fig. 2). Crystals in isolated cells	Approx. 20 rows thin-walled, isodiametric cells	Approx. 10 rows elongated tangentially, smaller than cortical cells, adjacent to carpel wall	Radial in epidermis; radial and tangential in hypodermis. Isolated in outer cortex, cortex, and pith
7 11.x.57	,,	,,	,,	Parenchyma cells slightly enlarged	,,	,,
14 18.x.57	,,	3 or 4 cells wide—crystals in some cells	10–15 rows	Approx. 50 rows—decreased cell size—cells rectangular. Walls of isolated, single cells lignified (future sclereids) (Pl. 4, Fig. 1)	Walls of single isolated cells becoming lignified	Active in all tissues—especially outer pith
25 29.x.57	Cuticle thicker—radial and tangential division	,,	Single sclereids throughout tissue—crystals in outer cells	Approx. 80 rows—sclereids single or in groups. Largest groups (up to 10) near pith	Groups of sclereids prominent—large groups in outer pith	,,
33 6.xi.57	Cuticle thicker—cells becoming irregular—elongated radially	3, 4, or 6 rows—cells dense, elongated tangentially—size of epidermal cells	High proportion of sclereids—parenchyma cells half size of sclereids	Approx. 120 rows—parenchyma cells enlarging—rectangular cells radiating from enlarging sclereid groups	Parenchyma cells approx. half area of cortical cells in section. Sclereid groups enlarging	In outer cortex and in the cells surrounding sclereid groups—also epidermis and hypodermis

45 18.xi.57	Cuticle thicker—cells becoming irregular—elongated radially	Cells larger than epidermal cells	Parenchyma cells approx. half size of sclereids. Sclereid walls thick (Pl. 5)	Parenchyma cells and sclereids comparable size (Pl. 2, Fig. 1)—sclereid groups connected by radiating rows of rectangular cells—starch grains in parenchyma—air spaces forming	Parenchyma cells stretched tangentially—smaller than cortical cells	Main period of division finished—continuing in cells surrounding sclereid clusters
53 26.xi.57	,,	,,	Band of sclereids on outside of fruit. Approx. 75% of cells lignified (Pl. 6, Fig. 1)	Parenchyma cells enlarging—starch grains conspicuous. 50–70 sclereids in groups	Parenchyma cells becoming rounded, $\frac{1}{2}$–$\frac{1}{3}$ size of cortical cells—starch accumulating. 50–70 sclereids in groups	Continuous in epidermis, hypodermis, and outer cortex
Stage II						
143 24.ii.58	Cells irregular—pulled apart by cutical pegs on radial walls. Lenticels prominent (Pl. 6, Fig. 2)	3, 4, or 6 rows elongated tangentially—twice as wide as epidermal cells. Plastids prominent. Cell walls thick	Approx. 10–15 rows. Cells rounder, less dense than hypodermis. Walls thicker than cortical cells. Sclereid band separated into groups. Area of parenchyma and sclereids comparable in section. Plastids in parenchyma	Parenchyma cells 3 or 4 times size of outer cortical cells. Intercellular spaces small. Groups of sclereids (1–20 cells) scattered through cortex and separated by parenchyma cells elongated radially (Pl. 1, Fig. 2). Larger groups towards pith. No starch grains	Parenchyma cells smaller than cortical cells—walls thicker. Sclereid groups up to 50 or more cells in outer pith. No starch grains	Continuous in outer layers of maturing fruit, increase in fruit size without obvious increase in cell size and separation of sclereid groups

Sclereid formation began in the outer cortex 21 days from blossom and continued into Stage II; the appearance of this tissue, 42 days from blossom, is seen in Plate 6, Figure 1. This pattern of sclereid formation, also observed by Mitchell (1950) and Sterling (1954), is represented in Figure 4.

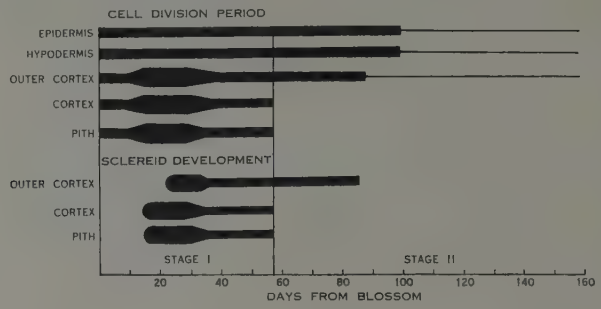

Fig. 4.—Diagrammatic representation to show period and distribution of cell division and sclereid formation in developing fruit; the relative amounts are shown by the width of the histograms.

(iii) *Physiological Observations.*—These data were obtained for the whole fruit during Stage I as separation of cortex and pith was not possible.

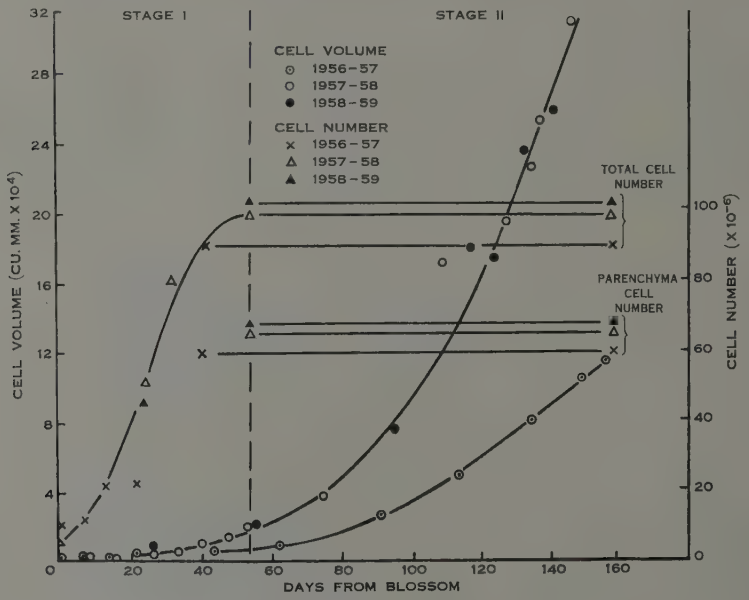

Fig. 5.—Changes in cell size and cell number in the developing fruit during three seasons.

(1) *Weight.*—Fruit weight increased from 0·1 g to approximately 12·0 g in the first season and to approximately 23·0 g in the other two seasons, dry weight increasing from 0·02 g to 3·0 g and to approximately 5·0 g respectively (Figs. 2 and 3).

(2) *Carbohydrate Content.*—Total sugars (mainly reducing sugars) and starch content on a dry- and fresh-weight basis decreased until the end of the main cell division period (28 days from blossom), and then increased during the rest of Stage I

TABLE 3

CHANGES IN CARBOHYDRATE CONTENT DURING STAGES I AND II IN FRUIT DEVELOPMENT

Days from Blossom	Total Sugars		Reducing Sugars		Starch	
	(mg/g dry wt.)	(g/fruit)	(mg/g dry wt.)	(g/fruit)	(mg/g dry wt.)	(g/fruit)
1956–57 season						
Stage I						
0	212·5	0·004	194·1	0·003	15·0	<0·001
21	70·3	0·02	50·5	0·01	5·1	<0·001
43	36·5	0·04	16·3	0·02	1·4	<0·001
63	81·8	0·28	68·7	0·24	2·3	0·01
Stage II						
91	144·3	1·52	123·6	1·31	75·1	0·80
113	171·8	2·65	160·6	2·48	73·8	1·14
133*	447·0	9·63	432·9	9·32	55·0	1·18
148	463·7	11·71	402·2	10·16	53·4	1·35
154	462·8	13·60	357·0	10·49	61·9	1·82
1957–58 season						
Stage I						
0	116·6	0·002	111·7	0·002	4·5	<0·001
25	40·4	0·02	34·1	0·02	3·0	0·002
53	91·8	0·38	72·8	0·30	22·1	0·09
Stage II						
74	203·3	1·55	186·8	1·42	81·9	0·62
109	330·5	6·47	304·6	5·96	76·8	1·50
124*	467·3	12·35	416·5	11·00	64·2	1·70
131	472·7	13·04	396·2	11·93	69·5	1·92
136	532·6	13·53	428·6	10·89	58·6	1·49
143	585·6	17·88	427·7	13·06	32·4	0·99
1958–59 season						
Stage I						
25	30·0	0·02	19·6	0·02	2·3	0·002
54	81·1	0·35	68·5	0·30	29·5	0·13
Stage II						
94	280·4	4·97	253·5	4·49	46·1	0·82
116*	438·6	10·53	381·0	9·15	65·8	1·58
123	449·5	11·16	390·0	9·68	70·5	1·75
130	524·5	13·15	413·0	10·35	70·1	1·76
137	586·4	15·96	416·7	9·83	40·1	1·09

* Commercial maturity.

as cell enlargement began (Table 3). Carbohydrates per fruit increased throughout, respectively attaining in the three seasons approximately 0·2 g, 0·4 g, and 0·4 g total sugar, 0·1 g, 0·3 g, and 0·3 g reducing sugar, and 0·01 g, 0·09 g, and 0·13 g starch (Table 3, Fig. 6).

(3) *Nitrogen.*—Changes in nitrogen level are shown in Figures 6 and 7. Total and protein nitrogen expressed on a dry- or fresh-weight basis decreased with time. Nitrogen increase per fruit was rapid during this period, with protein nitrogen the larger fraction during the cell division period. Protein nitrogen per cell increased with cell surface throughout Stage I (Fig. 8).

(4) *Acidity.*—Titratable acidity dropped on a dry- and fresh-weight basis in all seasons, but increased on a "per fruit" basis (Fig. 6).

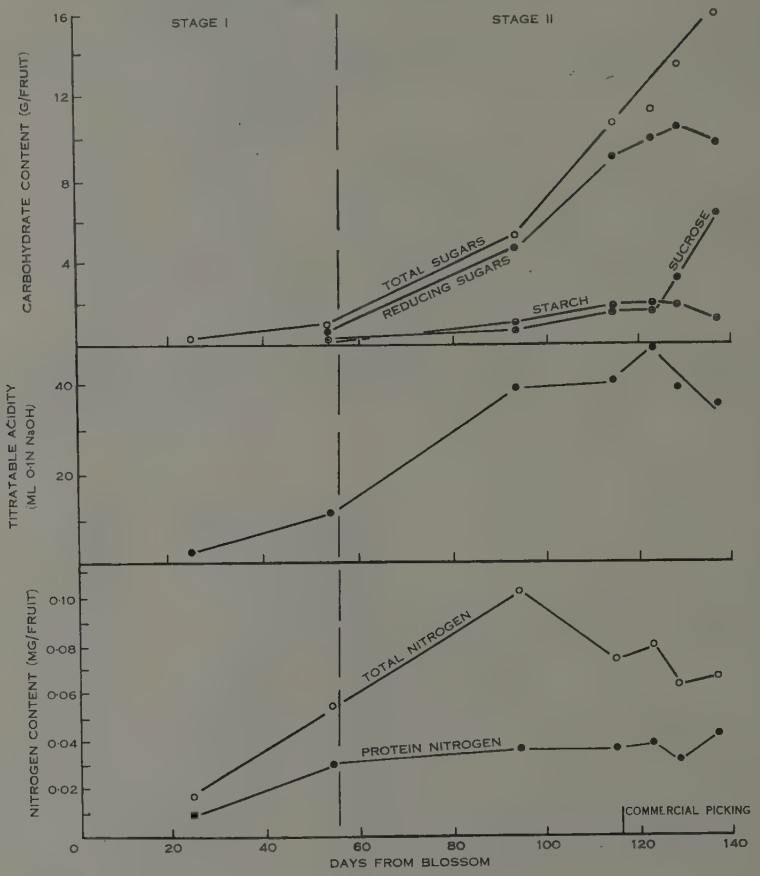

Fig. 6.—Changes in carbohydrate content, titratable acidity, and nitrogen content during the 1958–59 season.

(5) *Respiration.*—Respiration rate was very high per unit weight in the earliest stages of development. This fell to approximately 1000 mg CO_2/10 kg/hr by the end of Stage I and the rate per fruit rose steeply to reach approximately 3·0 mg CO_2/hr (Fig. 9).

(*b*) *Stage II—The Cell Enlargement Period*

Data for Stage II were collected for 98, 87, and 81 days respectively for the three successive seasons. The period from the end of Stage I to commercial maturity

was 77, 68, and 60 days respectively, picking being delayed in the first season with the hope that rain might fall to increase size.

(i) *Morphological Observations.*—Fruit increased in size at a rate slightly less than in Stage I until approaching commercial maturity, when a further decrease in the growth of the long axis, short axis, and cortex was seen (Fig. 3, Table 1). The cortex was approximately 60% of the radius of the maturing fruit. Differences in growth form were found in the later part of the three seasons: length and diameter increased proportionately in 1956–57, length increased faster than width in 1957–58, and diameter increased with little increase in length in 1958–59.

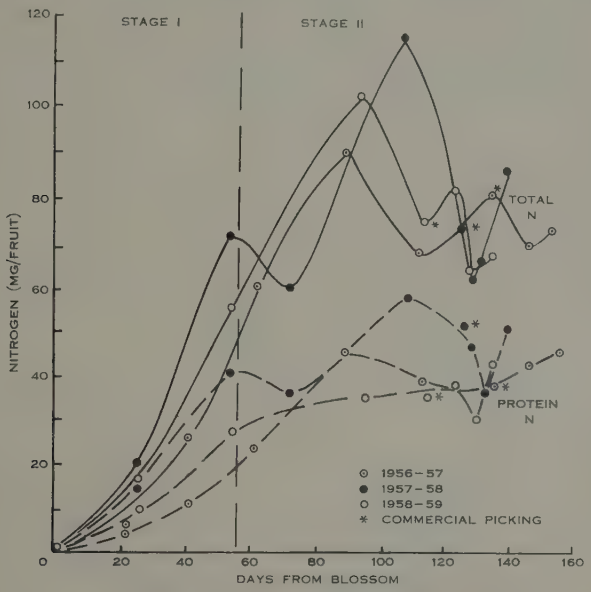

Fig. 7.—Changes in nitrogen content during three seasons.

Mitchell (1950) observed that the Bartlett pear ceased to grow during the eighth to twelfth week of development, this period corresponding to active growth of the embryo. Morphological change was not followed at close intervals during this period in the Bathurst pears, but the rate of increase of fresh weight at weekly intervals 8 to 12 wk from blossom in 1958–59 did not alter during this early part of Stage II. These pears, however, had only a small number of seeds.

(ii) *Anatomical Observations.*—Stage II was mainly a period of cell enlargement, increase in cell number continuing only in the epidermis, hypodermis, and the outer cortex (Fig. 4).

(1) *Anatomical Changes.*—Detailed anatomical changes occurring during Stage II are set out in Table 2. Cell division was associated with sclereid formation in the outer cortex up to 84 days from blossom; further increase in cell number occurred, and separated the outer band of sclereids into groups of sclereids in the mature fruit. Cell division was difficult to identify in the sectioned epidermis and

hypodermis as the fruit enlarged, but as cell size altered little with maturity, it was concluded that cell number increased. A surface view of the epidermis of the mature fruit showed the cells grouped into units separated by wide bands of cuticle; these cells were irregularly shaped and had thin walls indicative of more recent cell division.

Cell enlargement, already begun at the end of Stage I, continued at a greater rate in the early part of Stage II, with a further increase as the fruit matured. This rate was maintained as long as the fruit was left on the tree. At the end of sampling cell volume was 30×10^{-4} cu.mm. and 26×10^{-4} cu.mm. in 1957–58 and 1958–59 seasons and 12×10^{-4} cu.mm. in 1956–57 (Fig. 5). Cell shape differed in the second and third seasons, though size was comparable; cells in 1958–59 were longer and narrower than in 1957–58.

(2) *Structure of Mature Pear Tissue.*—Large groups of sclereids, made up of up to 50 or more cells as seen in section and concentrated in the outer cortex and

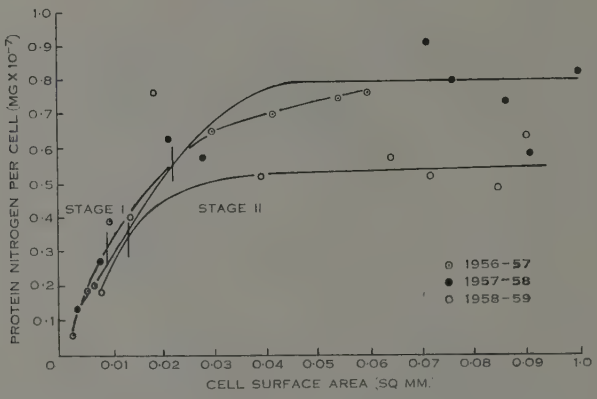

Fig. 8.—Relationship between protein nitrogen per cell and cell surface area during three seasons.

inner cortex–pith zone, were a feature of mature pear tissue. These sclereids were surrounded by thin-walled, radially elongated cells, and when the groups were close together these elongated cells became continuous and the true parenchyma divided into pockets of tissue. The cells of the outer cortex and pith were comparable in size, and were about half the size of cortical cells. Little variation was observed in parenchyma cell size within each tissue when complete sections were traced across the fruit. Intercellular spaces were small and total air space was calculated as 9% to 10% of the fruit. A value of approximately 7% was given for mature fruit by Marcellin (1954).

(iii) *Physiological Observations*

(1) *Weight.*—Fresh weight increased as long as the fruit remained on the tree, although the rate of increase was less after commercial maturity in 1958–59 (Fig. 2). The cortex contributed the greater part of the weight, changes in its growth being reflected in the weight of the whole fruit. When tissue separation was possible at the beginning of Stage II in 1957–58 and 1958–59, the cortex was 68·9% and 75·5%,

respectively, of the total fresh weight. It increased to 81·7% at commercial maturity and then to 84·6% at the end of sampling for 1957–58; in 1958–59 it increased to 83·8% and then remained constant. The core and peel contributed little to variation in fruit weight between seasons, and the percentage fresh weight of core and peel in the fruit was comparable. Increases in dry weights per fruit were generally similar to the increase in fresh weight with time, but the decreased growth rate following commercial maturity was more pronounced in the 1958–59 season (Fig. 3). The cortex was approximately 80% of the dry weight of the mature fruit and contained approximately 84% of the moisture in the mature fruit. The peel had slightly less moisture than the core. The percentage moisture content increased from approximately 82% to 84% in the cortex, from 76% to 81% in the core, and from 68%

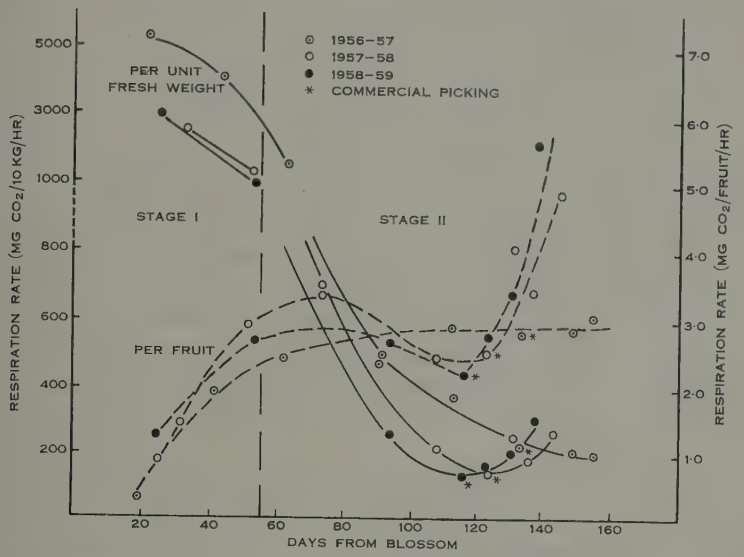

Fig. 9.—Respiration rate at 20°C per unit fresh weight and per fruit throughout development in three seasons.

to 77% in the peel during Stage II. The greatest rate of increase in the peel and core was early in this period.

(2) *Carbohydrate Content.*—Total sugar content, expressed per fruit or on a dry- or fresh-weight basis, increased throughout the life of the fruit on the tree (Fig. 6, Table 3). Increase in reducing sugars closely paralleled increase in total sugars, 90% of the total sugars being in this form at commercial maturity. Similar changes in reducing and non-reducing sugar content up to commercial maturity in developing Williams pears have been found by Ulrich and Thaler (1957). Increase in reducing sugars in fruit left on the tree after commercial maturity was slow, but non-reducing sugars increased considerably. Starch content per fruit increased beyond commercial maturity in 1957–58 and 1958–59 and started to fall by the end of the season; the level in 1956–57 was lower, and increased gradually throughout Stage II. On a dry- or fresh-weight basis starch reached a maximum early in Stage II

in 1956–57 and 1957–58 and then gradually decreased; a maximum was not reached until after commercial maturity in 1958–59 (Fig. 6, Table 3).

Hulme (1958) has summarized information available on the sugar content of developing and ripening pears; fructose, glucose, and sucrose make up the bulk of the total sugars, with fructose in excess of glucose, and glucose in excess of sucrose. Xylose, galactose, and sorbitol are also present. Data on the pattern of starch change during growth were not then available. Our work shows that as the starch begins to decrease after commercial maturity, reducing sugars also decrease and non-reducing sugars increase sharply (Fig. 6, Table 3) from 1·48 g per fruit at 123 days to 6·13 g per fruit at 137 days, though only a small portion of this (0·66 g) was accounted for by the fall in starch. After commercial maturity soluble carbohydrate continued to be imported by the fruit, and non-reducing sugars accumulated, probably mostly as sucrose (Hulme 1958).

(3) *Nitrogen.*—Total and protein nitrogen dropped sharply on a dry- and fresh-weight basis until commercial maturity was reached; the rate of decrease then slowed down. Considerable fluctuations were found in total nitrogen per fruit, mainly in the soluble fraction during Stage II (Fig. 7). Such fluctuations appeared to be real, as their absence in the carbohydrate determinations did not suggest sampling errors. Nitrogen per fruit increased in the first half of Stage II and then showed a general decrease in the second half. It is possible that nitrogen was being transferred to other areas of the fruit at the expense of the cortex, e.g. some nitrogen could have gone to the seeds or the peel. The same trends were observed in the three seasons, however, even when, as in the first season, the peel was included with the flesh sample. There was no relationship between the protein nitrogen and the soluble nitrogen level and the changes in the two forms of nitrogen were not comparable.

Protein nitrogen per cell did not increase with cell size during Stage II, the early linear relationship shown in Stage I not being maintained. There was also little further increase in protein nitrogen with increase in cell surface area (Fig. 8). This result was contrary to data obtained for apple fruits (Pearson and Robertson 1953), when protein nitrogen per cell increased considerably with increase in cell surface throughout the season. The fact that mature apple cells were approximately twice the size of mature pear cells may be concerned with the difference in behaviour.

(4) *Acidity.*—Titratable acidity fell on a dry- and fresh-weight basis as the fruit matured; it increased per fruit until commercial maturity and then fell (Fig. 6). A fall in total acids in Williams pears just prior to commercial maturity was observed by Ulrich and Thaler (1957), who showed that much of the change was due to the decrease in quinic acid.

(5) *Respiration.*—Trends in respiration rate were similar in 1957–58 and 1958–59, showing an increased rate in fruit left on the tree after commercial maturity; there was no such rise in 1956–57, a very dry year, and the data are not considered typical. Respiration rate dropped to approximately 150 mg CO_2/10 kg/hr at commercial maturity and increased to approximately 300 mg CO_2/10 kg/hr by the end of sampling (Fig. 9). The rate per fruit fell prior to commercial picking to approximately 2·3 mg CO_2/hr and then increased to approximately 5·3 mg CO_2/hr.

The sharp rise in respiration rate occurs at the same time as the rise in non-reducing sugars in the fruits (cf. Figs. 6 and 9 for the 1958–59 season) and introduces the possibility that there might be some causal relationship. There is some evidence that about this time (Rowan, unpublished data) a fall also occurs in the hexose monophosphate. At present there is insufficient evidence to suggest connexions between respiration rate and the carbohydrate imports and synthetic pattern, but it suggests that further investigation of the phosphorylated compounds might be of interest.

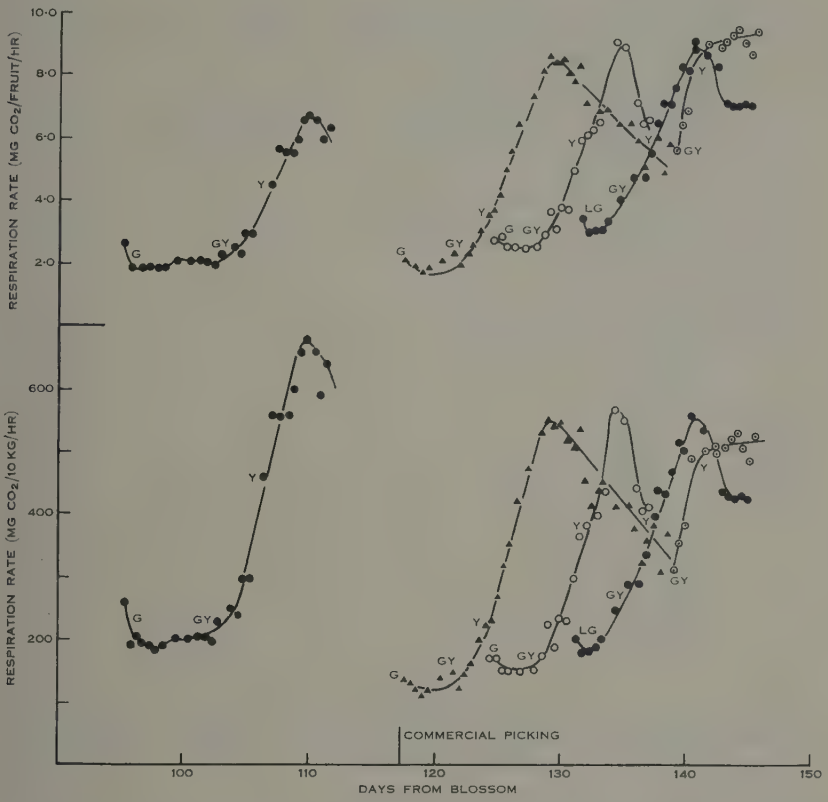

Fig. 10.—Respiration rates at 20°C per unit fresh weight and per fruit following removal from the tree at successive samplings in the 1958–59 season. Changes in skin colour are represented by: *LG*, light green; *G*, green; *GY*, green-yellow; *Y*, yellow.

(c) *Post-harvest Physiological Changes*

Pear ripening is associated with distinctive skin colour change, softening of tissue (Table 1), and a very marked climacteric rise in respiration. This rise is spectacular in fruit ripened off the tree. Tissue softening is associated with pectin changes and apparent thinning of the walls of parenchyma cells but with no change in the size or with no alteration in the size or number of the sclereids (Sterling 1954).

TABLE 4

CHANGES OCCURRING DURING RIPENING AT 20°C FOLLOWING STORAGE AT 0°C

Days of Ripening at 20°C	Average Wt. of 10 Fruit (g)	Weight Loss (%)	Moisture Content of Flesh (%)	Pressure $\frac{5}{16}$ In. Plunger	Colour	Total Nitrogen (mg)	Protein Nitrogen (mg)	Total Sugars (mg)	Reducing Sugars (mg)	Starch (mg)	Titratable Acidity (ml 0·1N NaOH)
						(per g dry wt.)					
1957–58 season											
Removal I—*Ex* cold 25.ii.58 (20 days at 0°C)											
1			84·1	16·1	Green	3·6	1·8	527·2	413·7	3·6	1·8
3			83·6	6·2	Green-yellow	3·3	1·9	537·1	439·1	1·5	1·9
6			84·2	< 3·0	Yellow	3·1	1·7	552·9	513·0	0·5	2·3
8			82·8			3·3	1·8	558·6	518·8	0·5	2·0
10			83·1			3·6	1·8	588·6	531·6	0·4	2·6
13			84·0			3·5	2·0	569·3	557·1	0·4	2·6
Removal II—*Ex* cold 13.iv.58 (67 days at 0°C)											
1			83·6	13·5	Light green	3·2	1·8	580·3	521·8	—	2·1
2			84·1	10·0	Green-yellow	3·9	2·0	550·2	490·0	0·6	2·4
3			83·7	5·1	Yellow	3·7	2·0	572·9	489·8	0·8	2·2
4			83·6	< 3·0		3·4	1·9	580·4	465·1	0·5	2·3
5			83·7			—	—	591·3	481·1	0·9	2·2
8			83·1			4·1	1·9	580·0	490·1	0·5	2·8
Removal III—*Ex* cold 13.v.58 (97 days at 0°C)											
0			83·6	13·4	Light yellow	4·0	1·9	566·1	487·3	1·0	1·9
2			84·0	5·9	Yellow	3·9	1·9	588·8	491·9	0·7	1·9

1958–59 season											
Removal I—*Ex* cold 4.iii.59 (22 days at 0°C)											
0	151·9	0·5	84·0	17·4	Green	3·4	1·7	537·1	471·9	3·8	1·7
2	151·9	0·9	84·2	16·0	Green	3·6	1·7	527·0	437·9	<0·5	1·5
5	149·9	1·7	84·8	< 3·0	Yellow	3·8	2·0	534·9	442·7	<0·5	2·0
6	150·6	2·3	85·2			3·5	1·9	537·2	444·9	<0·5	1·8
7	150·2	2·4	84·9			3·8	2·0	554·2	452·9	<0·5	2·0
10	148·3	3·2	85·0			3·9	1·9	556·1	460·4	<0·5	2·0
Removal II—*Ex* cold 3.iv.59 (61 days at 0°C)											
0	151·4	0·7	84·4	17·3	Light green	2·9	1·8	525·1	474·3	4·3	1·5
1	151·4	0·9	84·6	16·1	Green-yellow	2·8	1·9	528·7	470·1	3·3	1·5
2	153·0	0·8	84·2	8·5	Yellow	2·9	1·9	546·2	468·1	2·7	1·4
3	150·3	1·3	84·3	4·5		3·8	2·0	546·9	443·7	1·7	1·9
4	151·4	1·8	85·0			3·4	2·0	554·3	454·7	1·0	1·8
5	149·4	2·0	84·9			3·7	2·0	550·3	435·5	<0·5	1·9
6	149·5	2·2	84·9			3·5	1·9	545·2	453·1	<0·5	1·8
Removal III—*Ex* cold 27.iv.59 (85 days at 0°C)											
0	153·5	0·7	84·8	14·3	Light yellow	2·9	1·7	546·2	458·8	0·8	1·3
1	151·1	1·1	85·3	12·3	Yellow	3·5	1·9	554·3	464·7	0·6	1·3
2	152·4	1·4	84·6	6·7		4·0	1·9	537·3	459·2	<0·5	1·3
3	152·0	1·5	84·9	4·7		3·9	2·2	544·6	462·9	<0·5	1·5
4	150·0	2·2	84·9	3·7		3·1	1·9	549·1	448·8	<0·5	1·3

(i) *Physiological Changes in Fruit following Removal from Tree.*—For each of the three seasons, fruits from each picking taken during the later part of development were ripened, and respiration was recorded continuously until breakdown occurred in the fruit. Initial respiration rate (approximately 100–200 mg CO_2/10 kg/hr) showed a slight decrease in the first 4 days after commercial picking in the three seasons; at the end of this time fruit colour was changing from green to green-yellow. Following this, the respiration rate began to rise sharply, and increased by approximately 500 mg CO_2/10 kg/hr at the end of 5 days. Soon after this rise began the fruit became yellow. The climacteric peak (approximately 600–700 mg CO_2/fruit/hr) was maintained for 2–3 days and then the rate fell in the next 3–4 days as the over-ripe condition was reached (Fig. 10).

A climacteric rise was observed in each of the samples following commercial picking, but the initial rate was slightly higher at each pick. The time for colour change from green to green-yellow prior to the climacteric rise shortened with longer maturity on the tree; in the final samplings the climacteric began on removal from the tree. Post-climacteric life of the fruit decreased at each picking. The levels of the climacteric peak in the commercial and subsequent pickings were comparable in the 1958–59 season; in the 1956–57 and 1957–58 seasons the level decreased slightly with each pick. Fruit from the 1958–59 sample taken prior to commercial picking and left to ripen gave the highest climacteric pick. Changes in respiration rate during the ripening off the tree in 1958–59 are shown in Figure 10.

(ii) *Physiological Changes after Storage at 0°C.*—In the first season ripening changes for the three removals at 20, 67, and 97 days' storage at 0°C were followed for 13, 8, and 2 days respectively. In the second season removals were made at 22, 61, and 85 days and changes were followed for 10, 6, and 4 days (Table 4). Ripening time and the consumable life of the fruit both decreased with storage, the fruit being over-stored by the time of the third removal. The peel was green at the first removals (20, 22 days), light green at the second removals (67, 61 days), and light yellow at the final removals (97, 85 days). Fruit pressure dropped by approximately three units with the change from green to yellow at 0°C in both seasons, and fruit weight dropped by approximately 1 g during storage in the second season.

(1) *Weight.*—Total weight loss during ripening was approximately 3·2% at the first removal and 2·2% at the second and third removals in the 1958–59 season. This increasing weight loss with ripening needs to be considered in presenting analytical data for consecutive samples, especially if analyses are reported on a "per unit fresh weight" basis. Decrease in fresh weight, mainly due to moisture loss, causes the proportion of dry weight per unit fresh weight to increase with time, so that it is necessary to apply a correction to make the data found on a fresh-weight basis comparable for all samples (Martin 1937; Kidd *et al.* 1940). Actual analyses based on unit dry weight, as in the present data, are likely to be more comparable.

(2) *Carbohydrate Content.*—Total sugar content remained fairly steady on a dry-weight basis within each removal and did not alter with time in storage. Reducing sugars per gram of dry weight had increased at the first removal of the 1957–58 season, but remained steady in the other two removals in both seasons. Tindale, Trout, and Huelin (1938) reported an increase in sucrose on a fresh-weight basis during ripening. Starch disappeared from the fruit during storage, dropping from

approximately 65·0 mg per g dry weight at commercial maturity to approximately 4·0 mg by the first removal. Starch practically disappeared from the fruit during ripening. Martin (1937) reported that an increase in total sugars occurred in ripening Bosc pears which could not be accounted for by decrease of alcohol-insoluble residue, and showed that sorbitol decreased during the same period in quantities large enough to account for the increase in total sugars due to the associated increase in fructose. Kidd *et al.* (1940) obtained similar data on sorbitol in Conference pears during storage.

(3) *Nitrogen.*—Changes in nitrogen, carbohydrate, and acidity level per gram of dry weight during ripening are given in Table 4. Total nitrogen on a dry-weight basis fluctuated, mainly owing to the soluble fraction, the level of protein nitrogen showing little variation among samples.

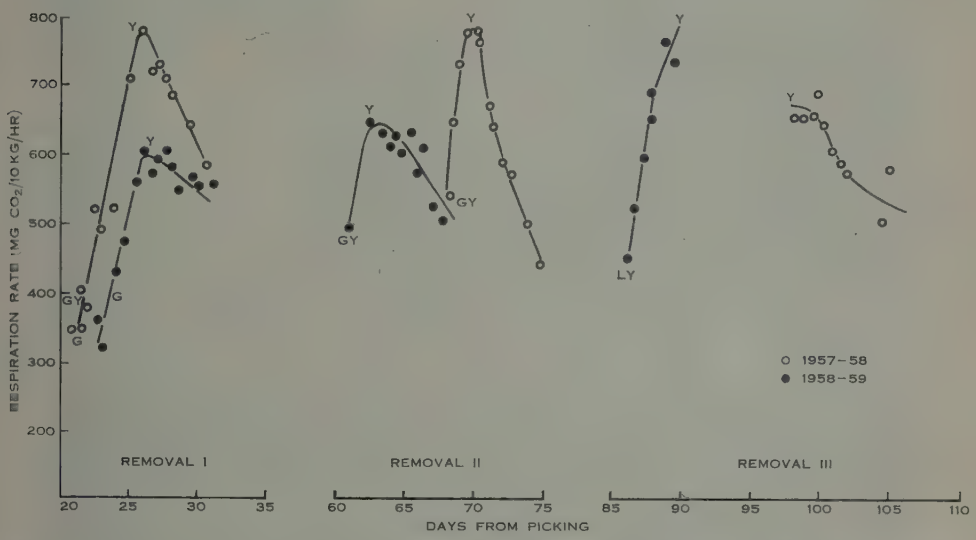

Fig. 11.—Respiration rate during ripening at 20°C after period of storage at 0°C in two seasons.

(4) *Respiration.*—A climacteric rise in respiration with change in colour from green to yellow closely followed removal of the fruit from storage; the climacteric peaks for the first and second removals in both seasons were slightly higher than during ripening at commercial maturity and the peak increased with time in storage (Fig. 11). The typical climacteric form was not found in the fruit in the third removal, which was over-stored and therefore did not ripen normally.

IV. Acknowledgments

I wish to express my sincere gratitude to Dr. R. N. Robertson for his continual interest in this investigation. Thanks are due also to Mr. J. Holbeche and Mr. C. Mort for their cooperation in supplying fruit from the Bathurst Experiment Farm, New South Wales Department of Agriculture; Mr. J. Smydzuk, Miss M. Woodham, and Miss B. Grimmond for analytical and technical assistance; and Mr. P. R. Maguire for the photography, with the exception of Plate 4, Figure 2, Plate 5, Figure 1, and Plate 6, Figure 2.

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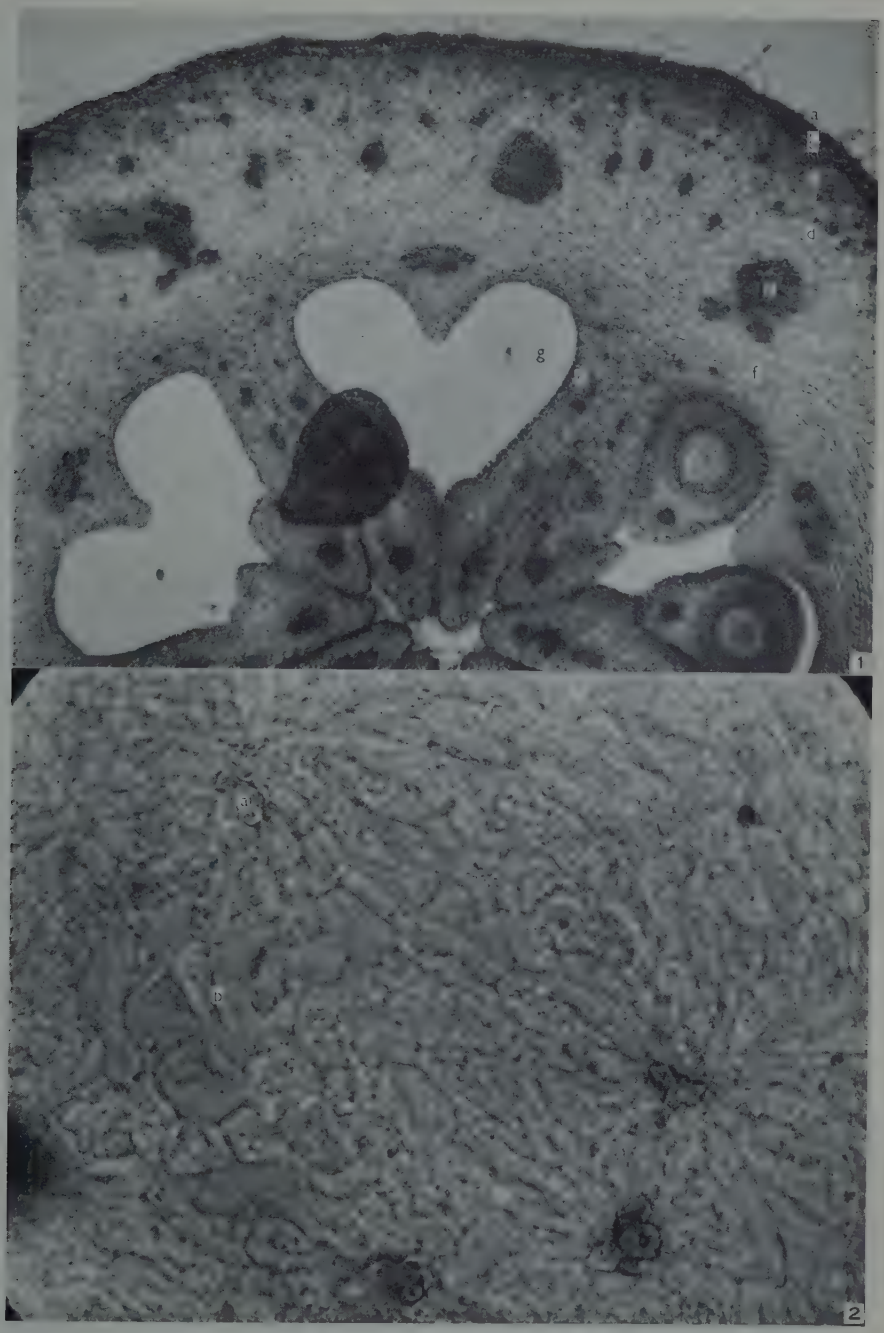
CHANGES IN THE DEVELOPING FRUIT OF THE PEAR



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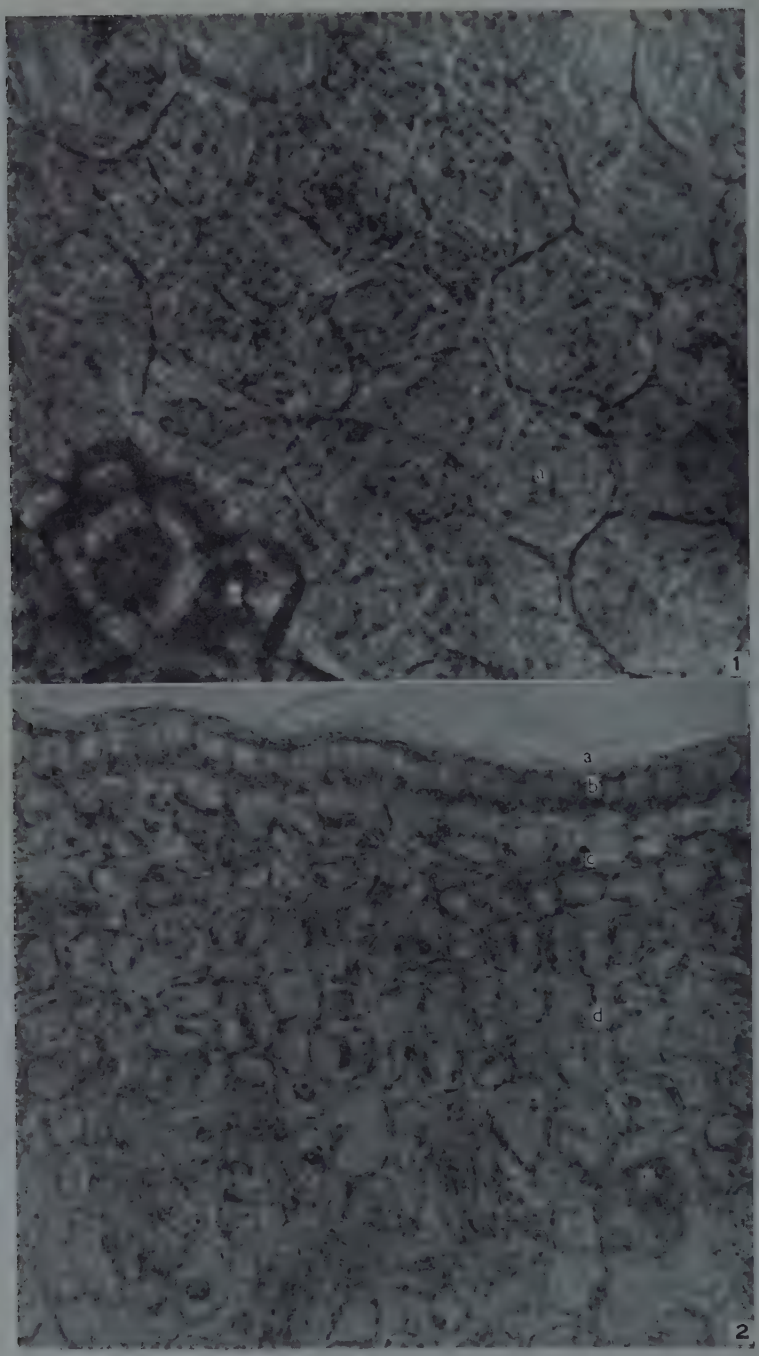

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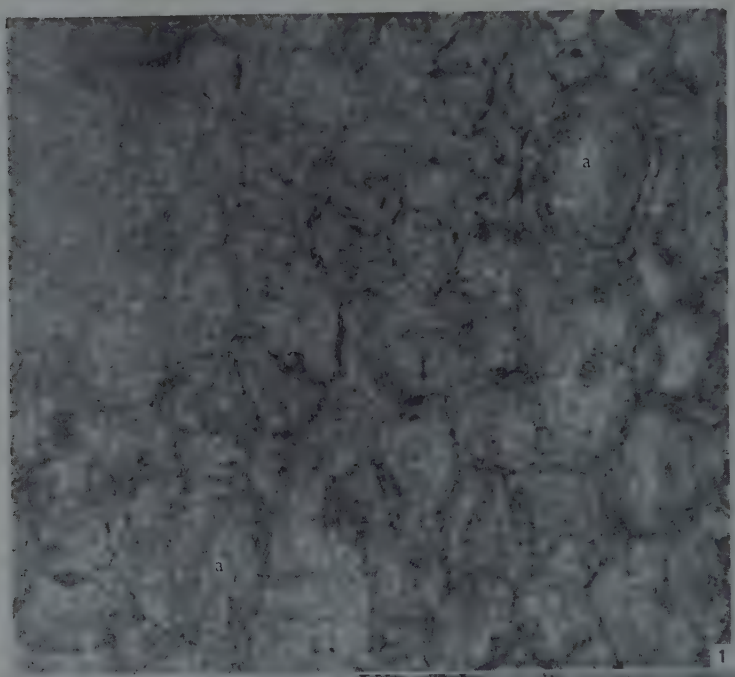

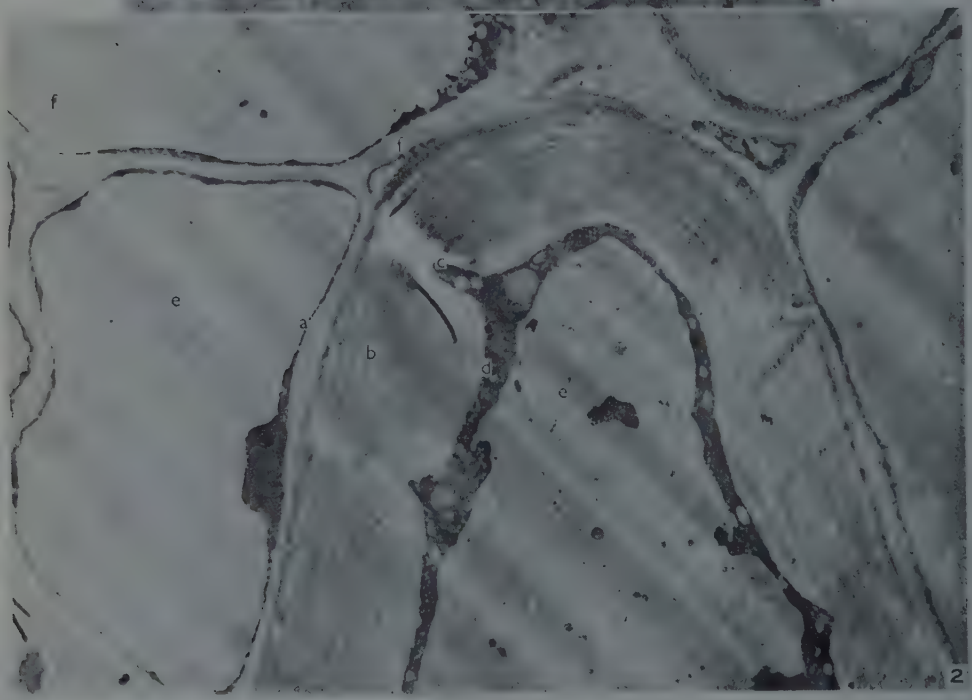

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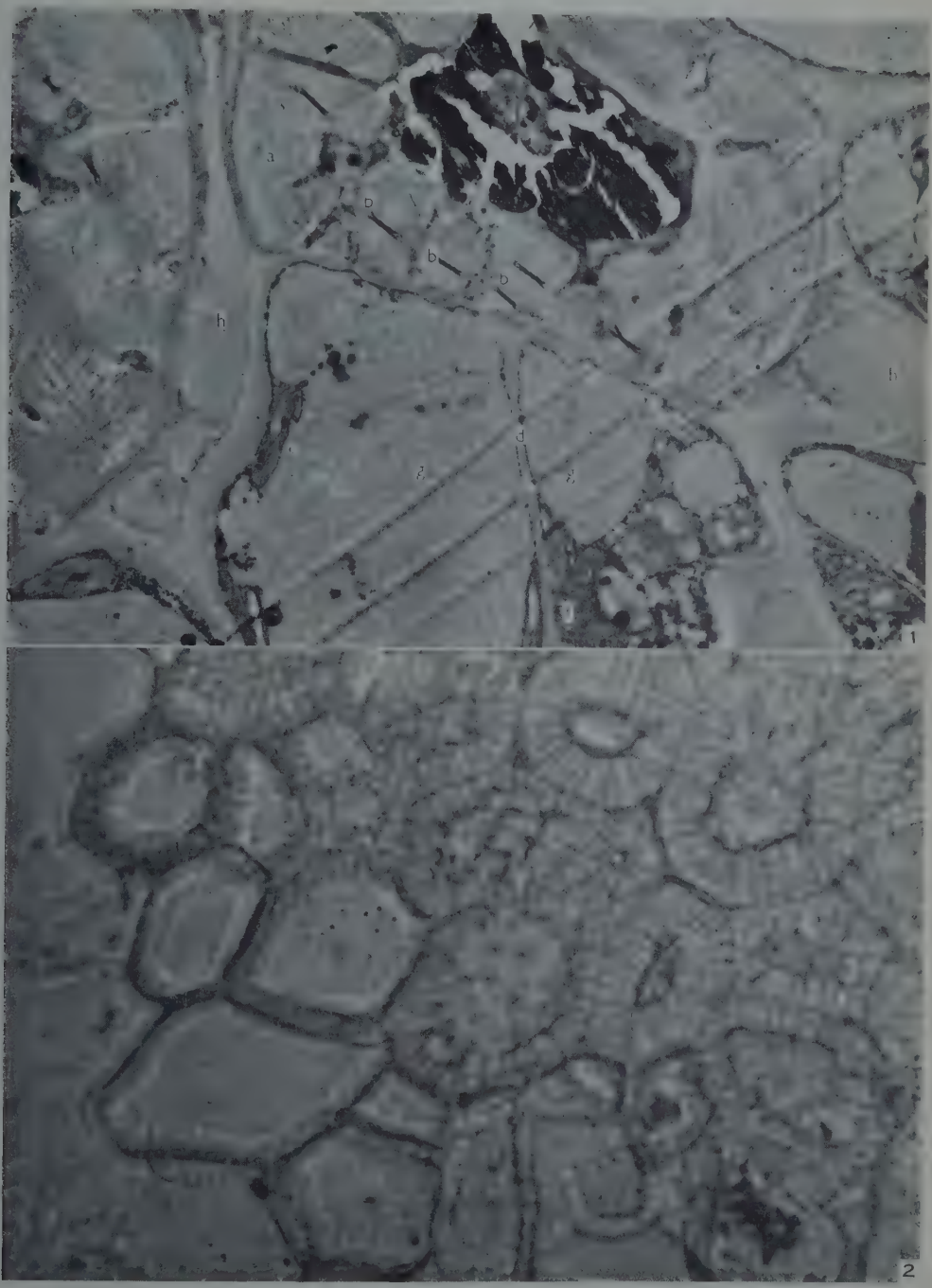

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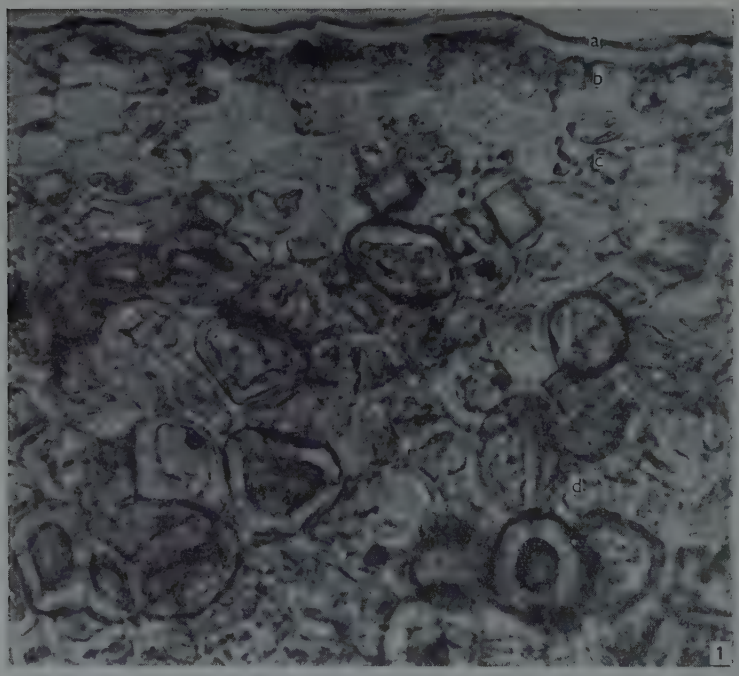
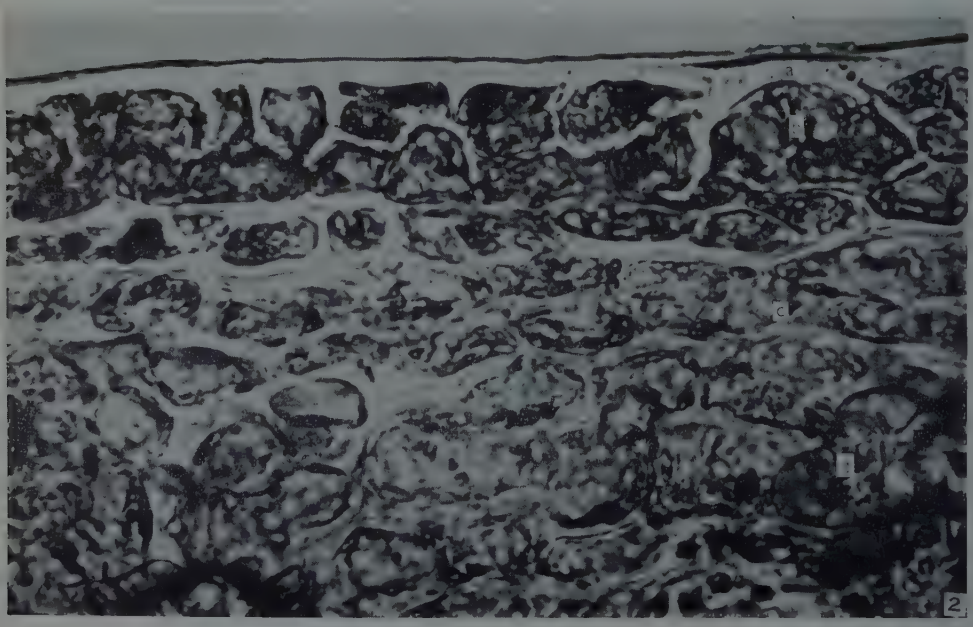




Explanation of Plates 1–6

Plate 1

Fig. 1.—Transverse section of ovary at blossom. Hand section. ×38.

a, epidermis+hairs
b, hypodermis
c, outer cortex } flesh
d, cortex }
e, ring of vascular bundles separating cortex from pith
f, pith
g, loculus of carpel } core
h, carpel wall }
i, ovule

} ovary wall

Fig. 2.—Transverse section of mature cortical tissue showing relative size of sclereids (*a*) and parenchyma cells (*b*). Sclereid groups are surrounded by thin-walled cells elongated radially. Parenchyma cells contain starch grains. ×75.

Plate 2

Fig. 1.—Transverse section of cortical tissue showing comparable size of parenchyma (*a*) and sclereid cells (*b*) at the end of Stage I. × 330.

Fig. 2.—Transverse section of outer ovary wall at blossom. ×330. *a*, Cuticle; *b*, epidermis; *c*, hypodermis; *d*, outer cortex with indications of cell division; *e*, formation of vascular network.

Plate 3

Morphological changes occurring during Stage I of growth. ×1.

Fig. 1.—Blossom.

Fig. 2.—After 21 days.

Fig. 3.—After 43 days (end of Stage 1).

Plate 4

Fig. 1.—Formation of sclereids in cortex 14 days from blossom. Walls of isolated cells becoming lignified (*a*) in actively dividing tissue. ×330.

Fig. 2.—Electron micrograph showing an early stage in sclereid formation. Material fixed in 1% osmium tetroxide and embedded in "Araldite". ×3300. *a*, Parenchyma cell wall; *b*, thickened wall of sclereid; *c*, pits in wall; *d*, cytoplasm withdrawn from wall; *e*, vacuole; *f*, intercellular space.

Plate 5

Fig. 1.—Electron micrograph showing structure of a well-developed sclereid. Material fixed in 1% osmium tetroxide and embedded in "Araldite". ×3000. *a*, Striated wall of sclereid; *b*, pits in wall of sclereid; *c*, disorganized cell contents of sclereid; *d*, parenchyma cell wall; *e*, chloroplast; *f*, cytoplasm; *g*, vacuole; *h*, intercellular space.

Fig. 2.—Sclereid formation at the edge of sclereid clusters at the end of Stage I. Fully-formed sclereids have very thick pitted walls and small lumen. ×500.

Plate 6

Fig. 1.—Transverse section of the outer tissues of fruit at end of Stage I. ×330. *a*, Cuticle; *b*, epidermis; *c*, hypodermis; *d*, outer cortex with high proportion of sclereids.

Fig. 2.—Transverse section of outer tissues of the mature fruit. Cuticular pegs are prominent between epidermal cells. ×540. *a*, Cuticle; *b*, epidermis; *c*, hypodermis; *d*, sclereids in outer cortex.

THE GAMETOPHORE AND SPOROPHYTE OF *MITTENIA PLUMULA* (MITT.) LINDB.

By Ilma G. Stone*

[*Manuscript received September* 5, 1961]

Summary

An account is given of the gametophore and sporophyte of *Mittenia plumula*, stressing those features which have not previously been described.

The moss has a highly refractive lenticular protonema. There is variation in the arrangement of leaves on sterile and fertile shoots, a shoot may develop one or more side shoots at its base, and hairs are present in the axils of leaves. The capsule has a few small stomata at the base, and the inner peristome consists of 28–32 processes.

The double peristome of *Mittenia plumula* originates from three concentric layers of cells, the two innermost layers of the amphithecium and the outermost layer of the endothecium. In all other members of the Bryales whose examination has included early development, the peristome has been found to develop from amphithecial tissue only.

The 16 outer peristome teeth are formed on the periclinal walls separating the two outer peristome layers, thickening being laid down on the inner walls of the outer layer, which is composed of 16 cells, and in adjoining positions on the outer walls of the middle layer, which is composed of eight cells.

The inner peristome processes are formed on the periclinal walls separating the middle from the inner peristome layer (the latter being endothecial in origin). Thickening is laid down on the inner walls of the middle layer at positions contiguous with the junctions of the anticlinal walls of the inner layer, which is composed of 24–28 cells. Extra processes usually develop where the anticlinal wall is part of the original quadrant wall of the embryo. A smaller amount of thickening is then contributed to the processes from the inner peristome layer.

Variations in the development of the inner peristome are discussed, and a comparison is made with development of the double peristome of other members of the Diplolepideae.

I. Introduction

Mittenia plumula (Mitt.) Lindb. was compared with *Schistostega pennata* (Hedw.) Hook. and Tayl. by early workers who considered that similarities existed between the gametophores of the two genera, and when, in addition, they were found to have the same type of highly refractive, lenticular protonema (Stone 1961), it was considered desirable to make a detailed examination of the gametophore and sporophyte of *Mittenia*.

No description of the development of the embryonic layers which give rise to the peristome or of the development of the inner and outer peristome of *Mittenia* has previously been recorded in the literature. Also, a close examination of the sporogonium reveals some features which differ from those in earlier descriptions of this moss.

* Botany School, University of Melbourne.

A similar detailed investigation of *Schistostega*, including serial sections of all stages in the development of the sporogonium which might reveal vestiges of peristome, or some other significant features, would be valuable for an assessment of the closeness of the relationship between the two genera.

General descriptions of *Mittenia plumula*, which is monotypic and the only member of the Mitteniaceae, have been given by various authors. The moss was first described from Tasmanian material by Mitten (1860), who named it *Mniopsis plumula*. Lindberg (1873) changed the name to *Mittenia plumula*. Muller (1901) recorded *Mittenia rotundifolia* for New South Wales but the Muller species has since been grouped with *M. plumula* Sainsbury (1955). Descriptions of the moss, with some figures, were given by Brotherus (1924) and Sainsbury (1955). There is also a description by Rodway (1914).

After submission to press of the paper on the protonema the author found an interesting paragraph on *Mittenia* by Goebel (1906). He collected the gametophore of *Mittenia* from the Black Spur Mt. near Melbourne, Vic., and compared it with *Schistostega*, referring to features of the leaf insertion and basal shoots.

II. Material and Methods

Mittenia plumula is not uncommon in the mountain ranges near Melbourne. Plants with mature sporophytes were collected in August 1960, and with young sporophytes in May and June 1961, from the Sherbrooke Forest.

The moss plants grow gregariously and in greatest profusion on the earth adhering to the upturned roots of fallen forest trees, but are also present on upturned pieces of root and earth in cavities under logs and on earth banks, often in association with *Fissidens* sp.

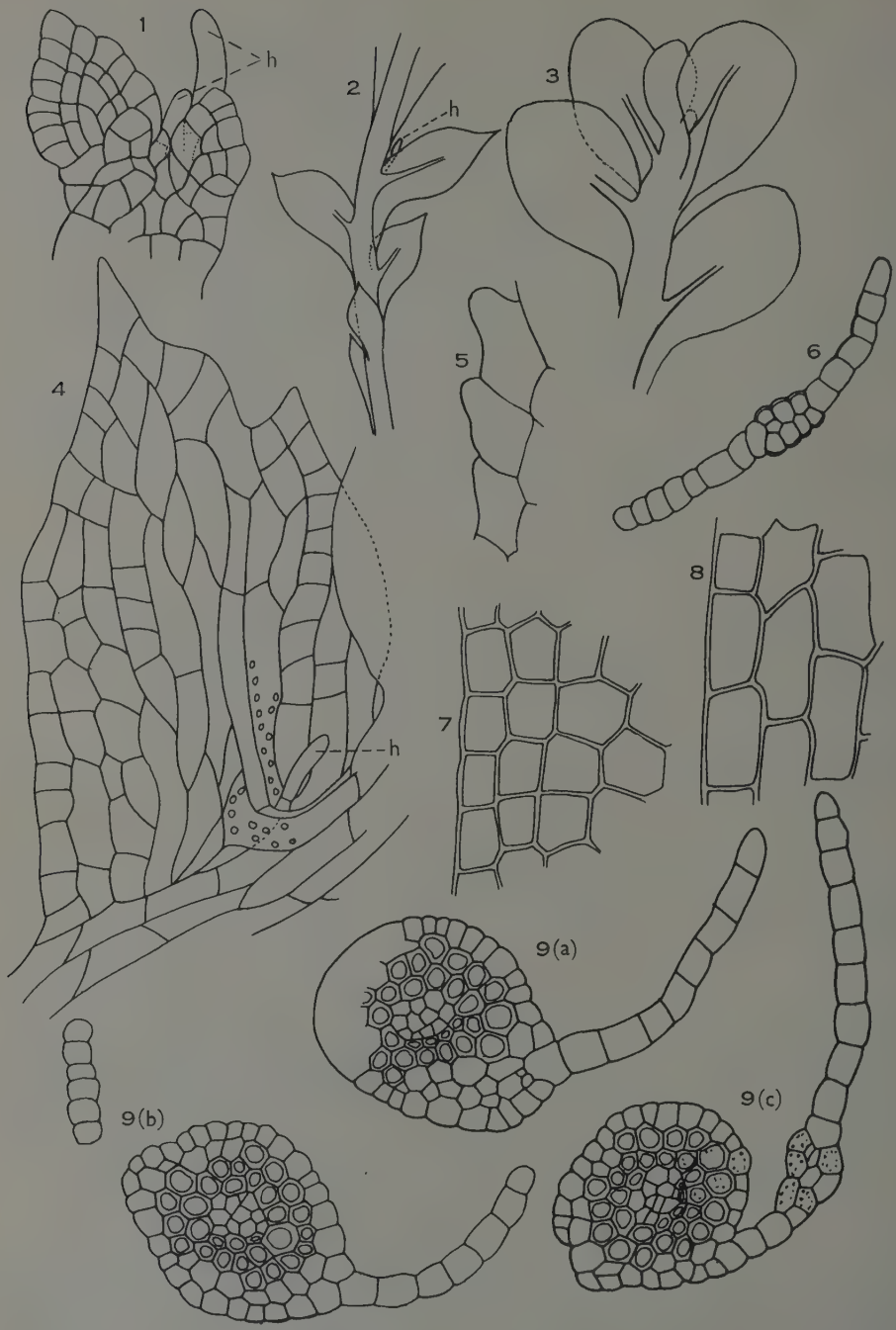
The plants were examined fresh and cleared with chloral hydrate. Gametophores and capsules at various stages of development were fixed in Craf 1 or F.P.A., the former being more satisfactory. They were dehydrated by the tertiary butyl alcohol–paraffin oil method and embedded in 54°C wax, following the method set out by Johansen (1940). Serial sections of stem, leaves, archegonial and antheridial shoots, and capsules were cut at 6, 8, or 10 μ and stained with iron alum haematoxylin counterstained with fast green, safranin and fast green, or Delafield's haematoxylin.

Hand sections of mature capsules which proved difficult to embed satisfactorily were mounted in 45% ethyl alcohol, which caused no movement of the peristome teeth.

Drawings were made with the aid of a camera lucida.

III. The Gametophore

The lenticular protonema (Fig. 20) and the gametophore of *Mittenia* obviously have different light optima, as the lenticular protonema persists all the year round in very shaded cavernous situations while gametophores are absent or sparse and sterile. Sterile and fertile gametophores are particularly numerous and cover the substratum where conditions are more favourable for their development. When a piece of substratum bearing numerous gametophores was accidentally subjected to



Figs. 1–9.—Gametophore of *Mittenia*. Fig. 1.—Apex of shoot with hairs (*h*). ×340. Figs. 2, 3.—Portions of vegetative shoots, showing bilateral insertion of leaves, and variation in leaf shape. Fig. 2. × *c.* 30. Fig. 4.—Leaf with bifurcation. ×210. Fig. 5.—

[*For continuation see opposite page*]

very weak light, the gametophores became pale, but before they decayed sent out numerous protonemal filaments with some gemmae from the leaf axils. From the thick underground-travelling rhizoids there was a great development of the lenticular protonema, which invaded the surface of the substrate and covered the decaying gametophores.

Richards (1932) discussed the fact that the optimum light conditions were different for the protonema and the mature plant of *Schistostega*.

The gametophores of *Mittenia* are generally less than 1 cm high and not quite vertical, but inclined at an angle to the substratum. The asymmetrical leaves vary in shape from broadly oval to oblong lingulate, the apices are bluntly pointed, shortly apiculate, or occasionally very rounded (Plate 1, Fig. 1, and Figs. 2–4, 15). Sometimes there is a slight bifurcation on the acroscopic side of the leaf (Fig. 4) and, although the marginal cells are usually smooth in outline (Figs. 7, 8), they are sometimes slightly crenulate as in Figure 5.

The lower leaves are small and usually nerveless while the upper leaves are more crowded and have a costa which ends above mid-leaf (Plate 1, Fig. 1). Figure 6 is a transverse section of a leaf showing the nerve. Figure 7 shows the surface and marginal cells from the mid region of a leaf. The cells are approximately isodiametric and 22–29 μ in diameter. They are slightly larger and not as thick-walled as those depicted by Sainsbury (1955) for *M. plumula* in New Zealand. Figure 8 shows cells from the same region of a longer leaf from a male shoot. They are more elongated than those in Figure 7.

There is considerable variation in the arrangement of leaves on the shoot. In the region of the growing point the leaves are radially arranged and have a transverse to oblique insertion (Fig. 1). This figure is similar to the apex of *Schistostega* figured by Goebel (1905).

In the mature vegetative shoot the leaves are displaced from this position and the shoot has a bilateral arrangement with the leaves standing in two rows (cf. *Schistostega*). The leaf has a vertical insertion of the larger basiscopic portion of the lamina which is decurrent with the stem, frequently until it reaches the level of the junction of the costa of the leaf directly below it (Figs. 2, 15). The smaller acroscopic region may have a vertical insertion or be partly free, but is usually attached obliquely to the stem, alternate leaves in a rank having this attachment on dorsal and ventral surfaces respectively (Fig. 2). Figure 9 (*a*)–(*c*) shows transverse sections of the insertion of a leaf on the stem: (*a*), insertion in the region of the costa; (*b*), insertion below; and (*c*), insertion above the costa.

The stem has a central core of very thin-walled cells (Fig. 9) which frequently break down in older parts of the stem. Resorption of cells in the central strand is

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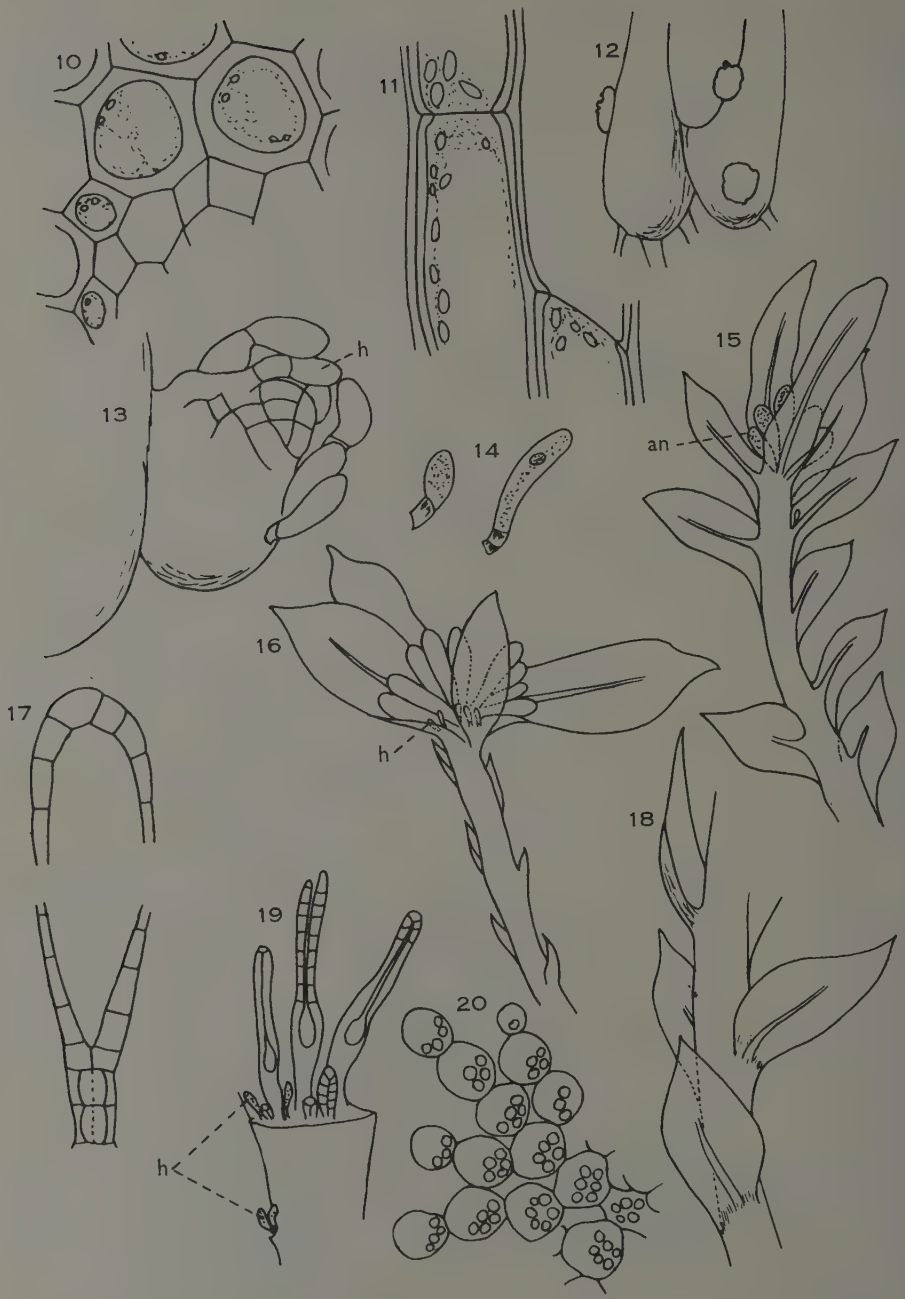
Figs. 1–9 (*Continued*)

Crenulate marginal cells of a leaf. ×450. Fig. 6.—Transverse section of leaf. ×210. Fig. 7.—Surface and marginal cells from mid region of leaf. Fig. 8.—Same, from a longer leaf of antheridial shoot. ×340. Fig. 9(*a*)–(*c*).—A series of transverse sections of insertion of a leaf on the stem: (*a*) insertion in the region of the costa; (*b*) below, and (*c*) above (*a*). ×210.



Figs. 10-20.—Gametophore of *Mittenia*. Fig. 10.—Transverse section of thickened cells of the cortical region, and thin-walled cells of the central strand, of stem. Fig. 11.—Longitudinal section of thick-walled cells of cortical region showing thin transverse walls. × *c.* 1000. Fig. 12.—Base of shoot with attached side shoot and basal buds.

[*For continuation see opposite page*]

similar to that figured for *Campylopus polytrichoides* by Lorch (1931). Between the epidermis and the central core there are two rows of elongated cortical cells with thickened longitudinal walls, but thin transverse walls (Figs. 10, 11). The walls of the epidermal cells are thicker than those of the central strand but not as thick as those of the cortical cells. Chloroplasts are larger and more numerous in the epidermal cells than in the cortical cells, and are absent from the central strand.

The thickening on the walls of the cortical cells appears to be of a different composition from the other cell walls of the stem. The epidermal cell walls, the primary walls of the cortical cells, and the thin walls of the central cells stain a deep pink with ruthenium red, the thickening of the cortical cells staining a pale pink. When safranin and fast green is used this thickening is a faint pink while all other walls stain a deep green. Lignin tests are negative and chlor–zinc–iodine stains the thickening of the cortical cells a faint blue violet and all other walls a deeper blue violet.

New shoots arise from buds which are formed at the bases of the stem, and in this way small groups of plants are produced; thus there is a branching of the stem, although it is generally described as simple. As the new shoot grows, the cells at the base, between it and the parent stem, become brown and the individual stems are easily separated. Figure 12 shows the base of a shoot with an attached side shoot, both of which show new basal buds. The tips of both stems had been injured and had given rise to protonemal filaments and gemmae. Figure 13 shows the apex of a young side shoot, with several hairs.

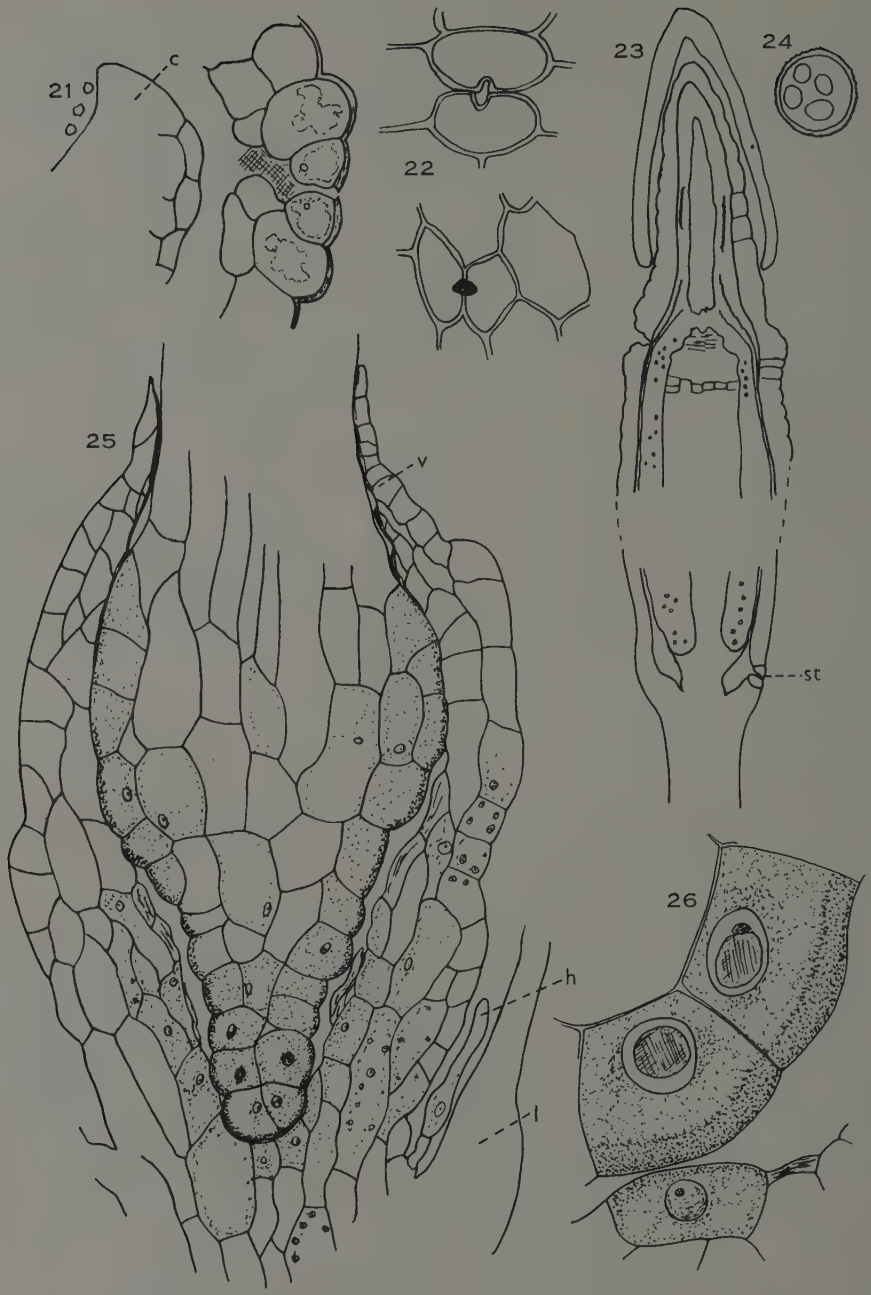
Male shoots which are about 7 mm in length appear to be of three types, and often arise from the base of an older shoot. Figure 15 shows the type with leaves arranged as on the vegetative shoot in the lower region, but the antheridia at the top are surrounded by longer leaves with a radial arrangement and oblique to transverse insertion. More rarely, the leaves on an antheridial shoot are radially arranged throughout the length of the stem.

Figure 16 shows a third type in which the stem is bare except for a few small scale-like leaves while the antheridia at the top are surrounded by five or more leaves which sometimes attain a length of 2 mm (cf. fertile shoots of *Schistostega*).

Antheridia are often very numerous and there may be 60 at all stages of development on one male shoot. No unusual features were observed in the development of the antheridium. Figure 17 shows the wall cells at the apex and the base of a cleared antheridium. Before dehiscence the cells at the apex are stretched thin and the operculum lifts when the spermatocytes are discharged in a sausage-like mass.

Figs. 10–20 (*Continued*)

× *c.* 50. Fig. 13.—Apex of side shoot with hairs. ×340. Fig. 14.—Hairs from leaf axil. ×340. Fig. 15.—Antheridial shoot with bilateral arrangement of leaves on the lower part of the stem; spatulate leaves surrounding antheridia. *an*, Antheridium. × *c.* 25. Fig. 16.—Antheridial shoot with scale-like leaves on the lower part of stem. × *c.* 25. Fig. 17.—Base and apex of cleared antheridium, showing cells of stalk and operculum. ×340. Fig. 18.—Radial arrangement of leaves on stem of archegonial shoot. Fig. 19.—Apex of archegonial shoot with leaves removed. ×80. Fig. 20.—Portion of lenticular protonema. ×340.



Figs. 21–26.—Sporogonium of *Mittenia*. Fig. 21.—Transverse section of base of sporogenous region, with stoma giving access to the air space. *c*, Columella. ×450. Fig. 22.—Two stomata in surface view. ×340. Fig. 23.—Longitudinal section of capsule. *st*, Stoma. ×80. Fig. 24.—Spore with chloroplasts. × *c*. 1000. Fig. 25.—Longitudinal section of foot of sporophyte embedded in the gametophore. The absorbing

[*For continuation see opposite page*]

The female shoots are 7–10 mm in length including the spatulate perichaetial leaves which may be 2 mm long. Figure 18 shows the radial arrangement and oblique insertion of leaves commonly found on the lower part of the female shoot. Some female shoots are similar to the male type shown in Figure 16 with a practically bare stem and a tuft of leaves at the apex.

Figure 19 shows young and mature archegonia at the apex of a shoot after removal of the surrounding leaves. The neck of the mature archegonium appears to be relatively short, with approximately 8–10 cells.

Hairs which are present on various parts of the gametophore consist of one or two tiny stalk cells with walls which turn brown early and a much larger terminal cell which has granular contents (Fig. 14). They are found among the antheridia and archegonia (Figs. 16, 19), at the shoot apex (Fig. 1), and on the buds at the base of a stem (Fig. 13). There may be 1–3 in the axils of leaves of both sterile and fertile shoots (Figs. 2, 15, 19), but these frequently shrivel at an early stage. The hairs on the *Mittenia* gametophore are similar in form to those figured by Ruhland (1924) for *Andreaea petrophila* Ehrh. and to that figured by Campbell (1895) in the axil of the leaf of *Sphagnum cymbifolium* Ehrh.

Goebel (1906) and Brotherus (1924) noted the presence of but did not illustrate paraphyses (hairs) on the gametophore. Paraphyses are not mentioned by Sainsbury (1955) or Rodway (1914), and the original description says "paraphyses nulles".

IV. Sporophyte

The mature capsule (Plate 1, Fig. 2), including the operculum and calyptra, is about 2·25 mm long. The slender tapering operculum has a cone-shaped base and the short pale green persistent calyptra is shed with the operculum.

The seta is 2–3 mm long, pale green, and has a small central core of thin-walled cells. The foot which penetrates the apex of the gametophore is short and swollen but tapered at the base and the cells in contact with those of the gametophore have large conspicuous nuclei and a concentration of granular material, which stains deeply with fast green and Delafield's haematoxylin, lining the rounded outer walls of the cells (Figs. 25, 26). Lorch (1931) noted and figured a similar accumulation of material in the absorbing cells in the foot region in other mosses. Figure 25 is a longitudinal section of the foot embedded in the gametophore. The vaginula which surrounds the base of the seta, and portion of a leaf with a hair in its axil are shown. The cells of the gametophore in contact with the foot usually have larger nuclei and denser contents than the other cells of the gametophore, and some concentration of granular material on walls adjoining those of the foot (Fig. 26). Very large compound starch grains are present in the vaginula, stem, leaves, and also in the calyptra.

Figs. 21–26 (*Continued*)

cells of the foot have large nuclei, and an accumulation of material lining their outer walls. Some of the adjoining cells of the gametophore are crushed. *v*, Vaginula; *l*, leaf. ×300. Fig. 26.—Two of the absorbing cells of the foot adjoining portion of the gametophore. The nuclei of the absorbing cells have a large densely stained portion and a nucleolus. × *c.* 1000.

The capsule of *Mittenia* has previously been described as having no stomata, but a careful examination after clearing shows that there are usually 2 or 3 small stomata on the lower part of the capsule, generally on a level with the lowest part of the archesporial tissue which extends almost to the base of the capsule (Fig. 23).

Figure 21 is a transverse section of the lower part of a capsule showing a stoma giving access to the air space. In section the guard cells are smaller than the adjoining cells. Figure 22 shows two stomata in surface view. The stomatal pores and the spaces behind them are frequently filled with brown material.

The peristome is double and inserted under the rim of the capsule. Plate 1, Figure 2, shows the cone-shaped base of the operculum, which is the region within which the short inner peristome processes lie. The long teeth of the outer peristome can be seen through the operculum in the upper tapering region.

Plate 1, Figure 3, and Plate 2, Figure 3, show the rim of the capsule and the double peristome from above and below. The 16 teeth of the outer peristome are curled outwards, which is the position taken up when conditions are moist, while the inner processes are bent inwards forming a dome over the spores.

These photographs and the one shown in Plate 2, Figure 1, which is portion of the peristome and exothecial cells detached from a capsule, show the inner and outer surfaces and also side views of the outer peristome teeth. There are prominent transverse bars on the ventral surfaces, more distant ridges on the dorsal surfaces of the teeth. The low basal membrane and processes of the inner peristome are also shown.

Plate 2, Figure 2, shows the inner peristome, which is about $\frac{3}{16}$ mm in diameter, with portion of the basal membrane detached from the capsule, and viewed with phase contrast.

The spores (8–10 μ in diameter) are green, with a few chloroplasts, and practically globose. The exine appears slightly rough when viewed under an oil immersion lens (Fig. 24). Sainsbury (1955) gives 10–12 μ as the diameter of the spores in the New Zealand specimens of the moss.

(*a*) *Development of the Embryonal Layers*

The regular division of the two-sided apical cell of the sporophyte takes place in the manner which is characteristic of the Bryales, as seen in Figure 46(*a*), which is a longitudinal section of the upper region of a young sporogonium. A transverse section of the tip appears as two approximately semicircular segments (Fig. 27). An anticlinal division in each of these segments produces a quadrant of cells (Fig. 28). As seen in transverse section each quadrant cell divides by one of two methods:

(1) The quadrant cell may be divided by a curved anticlinal wall which extends between the outside wall and one of the side walls producing a triangular and a four-sided cell, as seen in two quadrants in Figure 29. The four-sided cell then divides by a periclinal wall as shown in the two lower quadrants in Figure 30.

(2) The quadrant cell may be divided by a periclinal wall as in one quadrant in Figures 30 and 43(*a*). The outer of the two resultant cells then divides by an anticlinal wall.

In each case the ultimate result is the same and in transverse section there are four inner cells, the endothecium, surrounded by eight outer cells, the amphithecium. This is shown in Figure 43(*a*), except that divisions are not completed in one quadrant. These two embryonic layers proceed to divide independently and with great regularity, although there may be a lag in the divisions in some segments so that different sectors are at different stages of development as shown in most of the transverse sections of the developing capsule.

Figures 31–42 are a series selected from serial sections of a young sporogonium which shows the periclinal and anticlinal divisions in the two embryonic layers as seen in transverse section near the apex and at successively lower levels.

The eight amphithecial cells each divide by a periclinal wall (Fig. 44) so that two concentric layers of eight cells are formed. The inner layer of eight cells will be termed ring 1. Each of the cells of the outer layer divides by an anticlinal wall (Fig. 31) so that on completion of divisions there are 16 cells in the circumference.

This series of divisions is repeated, the 16 cells each dividing by a periclinal wall (Figs. 32, 43(*b*)), followed by an anticlinal wall in each of the outer daughter cells, producing 32 cells in the outer layer (Figs. 33–36). The inner layer of 16 cells will be termed ring 2.

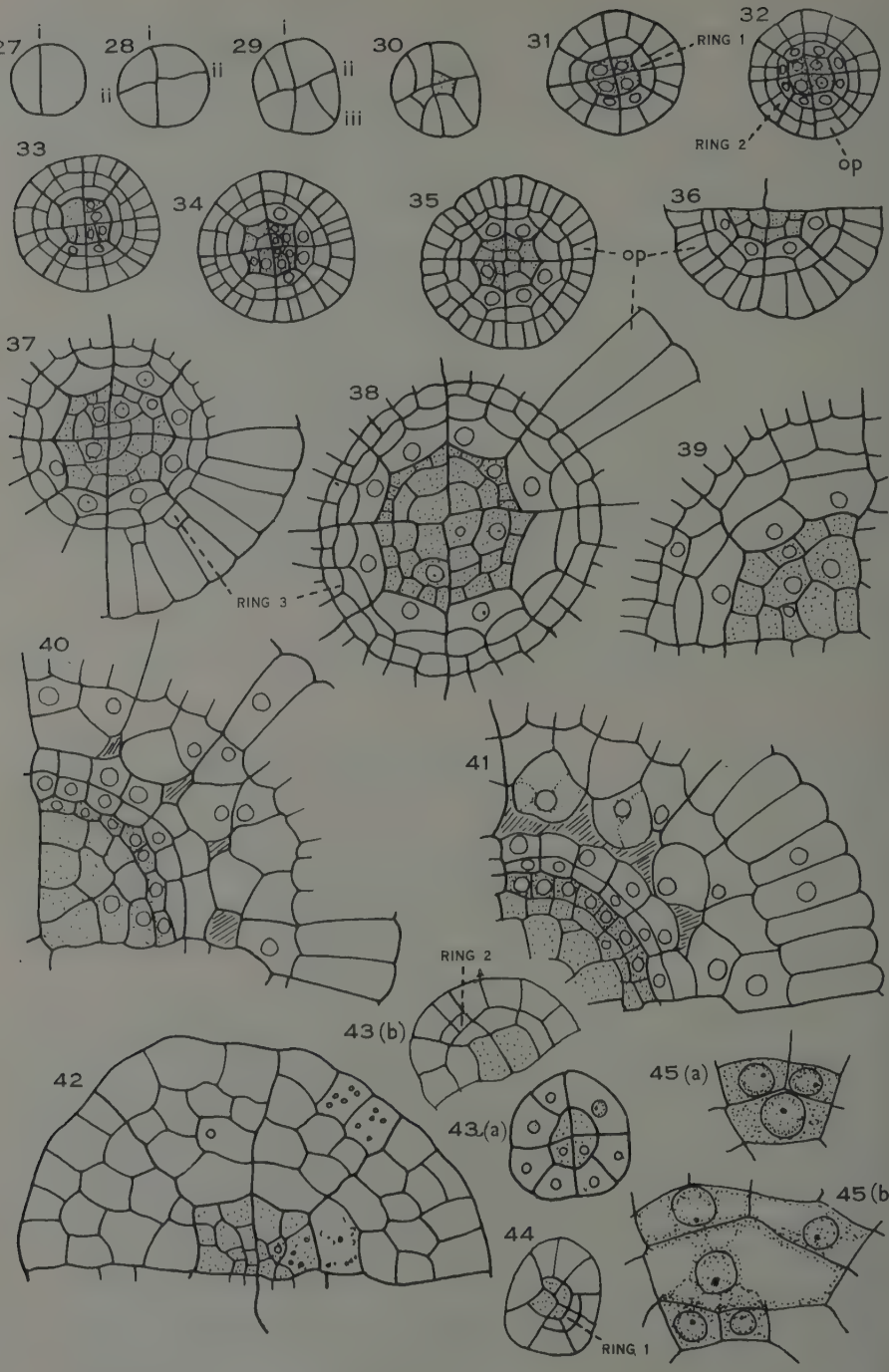
In the finely tapered portion of the opercular region, periclinal and anticlinal divisions do not proceed beyond this stage, and the operculum consists of the single outer layer of cells which later become thickened.

From just above the zone where the operculum separates from the rim of the capsule, downwards into the region of the spore case, each of the outer 32 cells of the amphithecium undergoes another periclinal division, two of which may be seen in Figure 37, which is a transverse section in the region just above the rim of the capsule. Figure 38, in the region of the capsule rim, shows these divisions completed so that there are two layers of 32 cells, the inner of which will be termed ring 3. Each of the cells of the outer layer divides anticlinally so that there are 64 cells in the outermost layer of the amphithecium in the region where rings 1, 2, and 3 are present (Fig. 39).

The delimitation of rings 1, 2, and 3 is seen in longitudinal section in the very young sporogonium shown in Figure 46(*a*)–(*d*), and Figure 47 shows the position of these layers in an older capsule.

A fourth ring of cells may later be cut off from the outer layer of the amphithecium but this is usually confined to the tier of smaller cells which constitute the region where the operculum splits away from the capsule at maturity. This is shown in longitudinal section in Figure 48 and in Plate 4, Figures 1 and 2. Plate 3, Figure 3, is a longitudinal section in which the fourth layer has also been cut off from the three lowest opercular cells.

Ring 1 remains eight cells in circumference in the tapered opercular region of the capsule, but in the lower cone-shaped part and in the region of the rim of the capsule, each of the cells of ring 1 may undergo a further anticlinal division, and at maturity this layer often consists of 16 cells. This anticlinal division is shown in one of the ring 1 cells in Figure 39 and in each cell in Figure 58.



Figs. 27–44.—Transverse sections showing development of the embryonic layers in the capsule of *Mittenia*. ×450. The amphithecium shows centripetal development, the

[*For continuation see opposite page*]

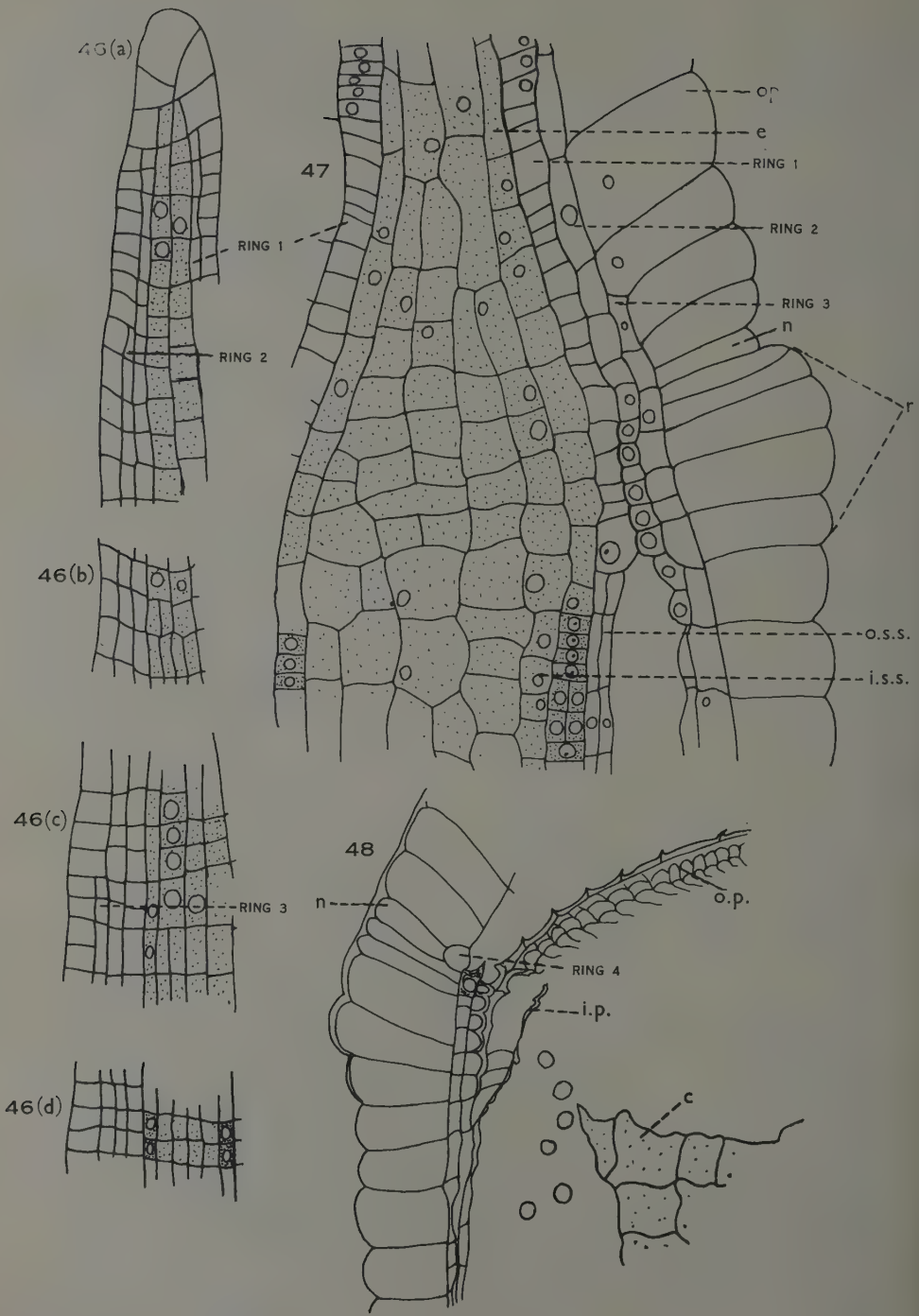
In the spore case region, the cells of ring 1 divide anticlinally and periclinally to produce two concentric layers of 32 cells, although there may be some irregularities in the divisions. The cells of the inner layer may undergo another anticlinal division. These two layers, which are usually termed the outer spore sac, are shown in transverse section in Figures 40 and 41 and in longitudinal section in Figure 47 and Plate 3, Figures 1–3.

Ring 2 with few departures remains 16 cells in circumference throughout the sporogonium. This layer plays a major part in the development of the air space in the spore case region. The cells become dilated and wedged between those of ring 3, while spaces develop between their radial walls and at their junctions with the outer tangential walls of the outer spore sac. The air space begins to develop at a very early stage in the lower spore case region and progresses upwards. Air space development is shown in Figures 40 and 41, which are transverse sections near the top of the sporogenous region, and in Plate 3, Figures 1 and 3, and Figure 47, which are longitudinal sections of portions of capsules.

The development of the air space in *Mittenia* differs from that in *Funaria hygrometrica* (L.) Sibth. in which short filaments extend across the air space between the wall of the capsule and the outer spore sac. Also the spore sac in *F. hygrometrica* consists of three layers of cells and the capsule wall of more than three layers (Campbell 1895). The features of the *Mittenia* capsule in the spore case region are like those of *Phascum cuspidatum* Schreb. and *Pleuridium subulatum* (Huds.) Rabenh. (both cleistocarpous mosses), in which, according to Campbell, the capsule wall consists of three layers of cells, the spore sac of two layers, and the air space between is complete, with no connecting filaments. This is probably also the case in the single peristomate moss *Pottia bryoides* (Dicks.) Mitt. (syn. *Mildeela*) (Limpricht 1890).

Figs. 27–45 (*Continued*)

endothecium (stippled) shows centrifugal development. Figs. 27–42.—A series of serial sections taken at successively lower levels from the tip downwards. Fig. 27.—Two semicircular segments at tip. *i*, First dividing wall. Fig. 28.—Quadrant of cells. *ii*, Second walls formed. Fig. 29.—Curved anticlinal wall, *iii*, in two quadrants producing a triangular and a four-sided cell. Fig. 30.—Delimitation of endothecium, from amphithecium. Fig. 31.—Ring 1, of eight cells, is the first layer delimited in the amphithecium. Fig. 32.—Ring 2, of 16 cells, is the second layer delimited in the amphithecium. *op*, Operculum. Fig. 33.—Curved anticlinal walls in endothecium. Fig. 34. Delimitation of outer layer of endothecium. Figs. 35, 36.—Amphithecial divisions complete for upper tapered part of capsule with 32 cells in the opercular layer. Fig. 37.—Section of lower cone-shaped region of the capsule. Figs. 38, 39. Sections in the region of the rim of the capsule. Ring 3, of 32 cells, is the third layer delimited in the amphithecium. Figs. 40, 41.—Sections of the upper part of the sporogenous region. Ring 1 has divided periclinally and anticlinally to produce the outer spore sac. The outermost layer of the endothecium is the archesporium, and the layer inside this is the inner spore sac. Air spaces are present between the outer spore sac and ring 2. Fig. 42.— Section of the seta. Fig. 43(*a*), (*b*).—Two sections of a young capsule showing early stages in the development of the amphithecium and endothecium, and delimitation of ring 1 and ring 2 in the amphithecium. Fig. 44.—First periclinal divisions in the formation of ring 1. Fig. 45(*a*).—Cells of rings 1 and 2 of Fig. 35 enlarged. The young cells have dense cytoplasm and a large nucleus. × *c.* 1000. Fig. 45(*b*).—Cells of Fig. 38 enlarged. The cytoplasm is vacuolated and strands pass from the nucleus to the regions where outer and inner peristome thickening will be deposited. × *c.* 1000.



Figs. 46–48.—Longitudinal sections of capsules of *Mittenia*. Figs. 46, 47 ×450. Fig. 46(*a*)–(*d*).—Sections of a very young capsule at successively lower levels, from the tip down to

[*For continuation see opposite page*]

The endothecium in *Mittenia* varies in the extent of its development at various levels down the capsule. The four cells of the endothecium divide by the first method (1) given above for the division of the original quadrant cells, and ultimately there is an inner group of four cells surrounded by a layer of eight cells, one large and one small outside each of the inner four cells (Figs. 33–35). The next divisions are anticlinal and occur in each of the larger cells of the outer layer, producing 12 cells in the circumference of the endothecium (Fig. 36). Further divisions do not appear to occur in this layer in the greater part of the opercular region.

The inner four cells again divide by the first method (1) (Fig. 37), producing another concentric layer outside the inner four. Lower down the capsule (Figs. 39, 40) a third concentric layer of cells is produced in the same manner. Divisions, however, are sometimes irregular.

Thus, in contrast to the divisions in the amphithecium, where the development may be said to be centripetal, the endothecium develops in a centrifugal manner. The first concentric layer of cells cut off from the endothecium is its outermost layer. It adjoins the first ring of cells cut off from the amphithecium, and the periclinal wall between, being the original wall separating the endothecium from the amphithecium, remains well marked throughout the development of the sporogonium.

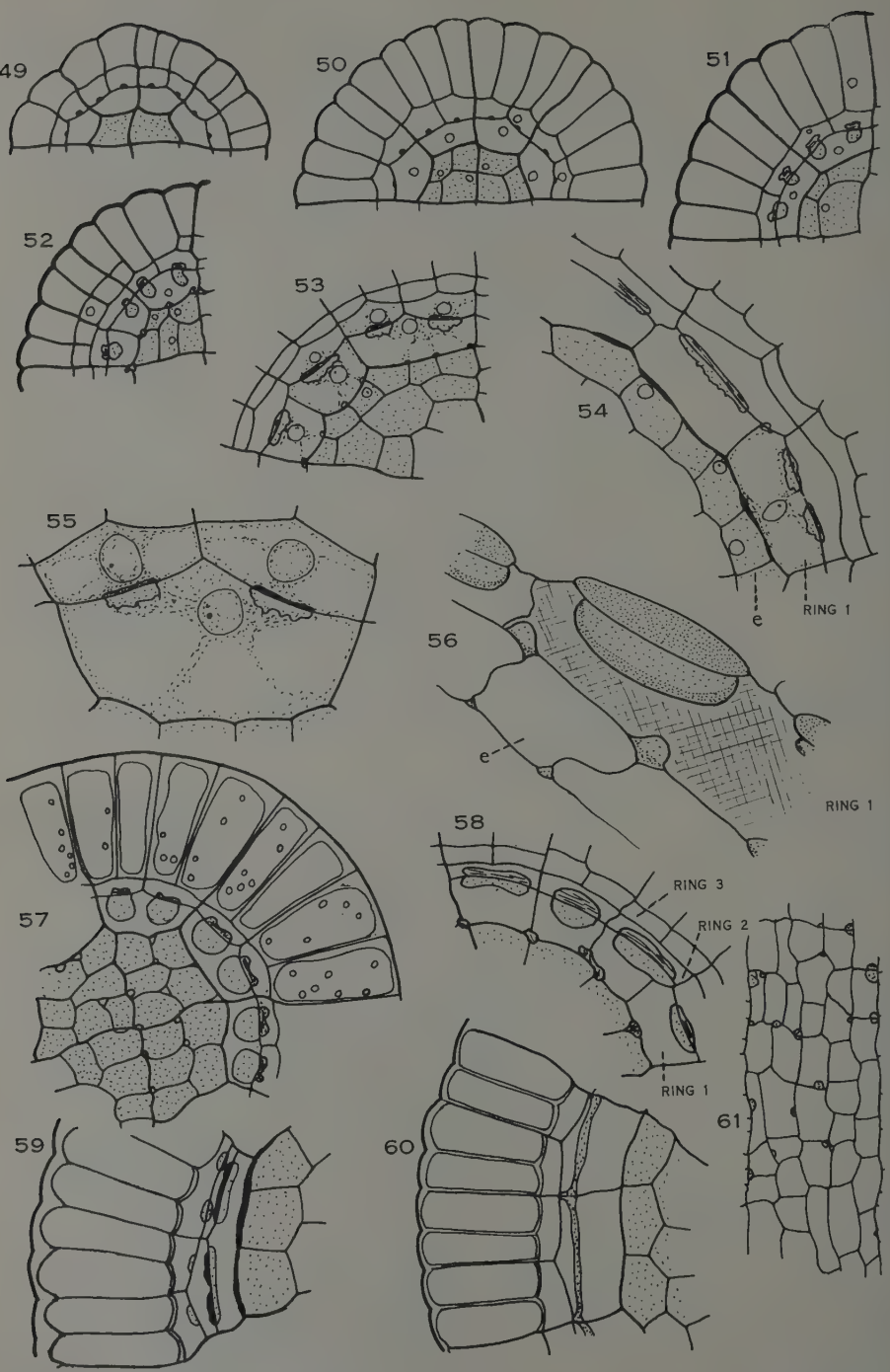
In the lower opercular region each of the four smaller cells in the outermost layer of the endothecium divides anticlinally, producing 16 cells. Figure 37 shows this division in three of the sectors. Figure 38 shows further anticlinal divisions in this layer and ultimately there are 24–28–32 cells in the outer layer which contributes to the inner peristome processes.

In the spore case region, this outer layer of the endothecium forms the sporogenous tissue, shown in Figures 40 and 41, and the cells of the second layer produced by the endothecium are divided by anticlinal walls to form the inner spore sac (Fig. 41). The cells internal to this layer constitute the columella.

Variation in the distribution of the transverse walls in the various layers is shown in Figure 47 and in Plate 3, Figures 1 and 2. The distribution of these walls in ring 1 and ring 2 in the opercular region determines the spacing of the bars on the dorsal and ventral surfaces of the outer peristome teeth. They are particularly numerous in ring 1, which is involved in the formation of part of both the inner and the outer peristome, and in which by far the greatest part of secondary wall deposition occurs. The transverse walls of ring 2 are closely spaced for a series of 6–8 cells where the basal portion of the outer peristome is attached to the capsule wall.

Figs. 46–48 (*Continued*)

the region where the archesporium develops. Development of the endothecium (stippled) and rings 1, 2, and 3 of the amphithecium are shown. Fig. 47.—Section of young capsule showing outer endothecial layer (*e*), which is the upward extension of the archesporium; ring 1, with numerous transverse walls; ring 2, with distant transverse walls in the opercular region, closely spaced transverse walls where the basal membrane of the outer peristome develops; and ring 3. *n*, Narrow cell in region where operculum breaks away from the rim (*r*); *o.s.s.*, outer spore sac; *i.s.s.*, inner spore sac. Fig. 48.—Capsule with mature peristome. *o.p.*, Outer peristome; *i.p.*, inner peristome. Ring 4 has been delimited in cell at line of dehiscence. ×340.



Figs. 49–60.—Transverse sections showing stages in the development of the peristome of *Mittenia*. Figs. 49–54 ×450. Figs. 49–51.—Outer peristome in the tapered region

[*For continuation see opposite page*]

The capsule of *Mittenia* is frequently curved and this may be accounted for by the fact that the cells on one side of the first longitudinal wall in the capsule develop slightly more than those on the other side. This is seen in Figures 36 and 38, but is more conspicuous in complete transverse sections of older capsules.

(*b*) *Deposition of Peristomial Thickening*

(i) *Outer Peristome.*—The 16 long outer peristome teeth are derived from the cells of ring 2 (16 cells in circumference) and ring 1 (eight cells in circumference). In the formation of the teeth a longitudinal strip of thickening is laid down on the inner periclinal walls of each of the cells of ring 2 (Figs. 49, 50, 69). This is soon followed by thickening laid down in two longitudinal strips on the outer periclinal wall in each of the eight cells of ring 1, in positions adjacent to the strips in ring 2, that is, in transverse section a group of three cells gives rise to two outer peristome teeth (Fig. 51).

The strips of thickening are widest at the base of the peristome and taper rather quickly in the cone-shaped region of the operculum, then very gradually to the almost filamentous tips.

Thickening also extends along the closely spaced transverse walls of ring 1 and gives rise to the trabeculae on the ventral side of the teeth (Plate 3, Fig. 3). Thickening along the more widely spaced transverse walls of ring 2 produces the bars on the dorsal side of the teeth and gives them a jointed appearance. These bars are shown in the longitudinal section in Figure 68, in portion of the mature tooth in Figure 72, and in Plate 2, Figures 1 and 3. In the radial longitudinal section shown in Figure 48, the bars on the dorsal and ventral surfaces of the teeth are seen as serrations with attached remnants of the transverse walls.

The young cells of the peristome layers have dense cytoplasm with conspicuous nuclei, each of which has one nucleolus (Fig. 45(*a*)). Later the nuclei and denser cytoplasm move close to the outer periclinal walls of the cells of ring 1, centred between the two regions of deposition in each cell, and to the inner periclinal walls

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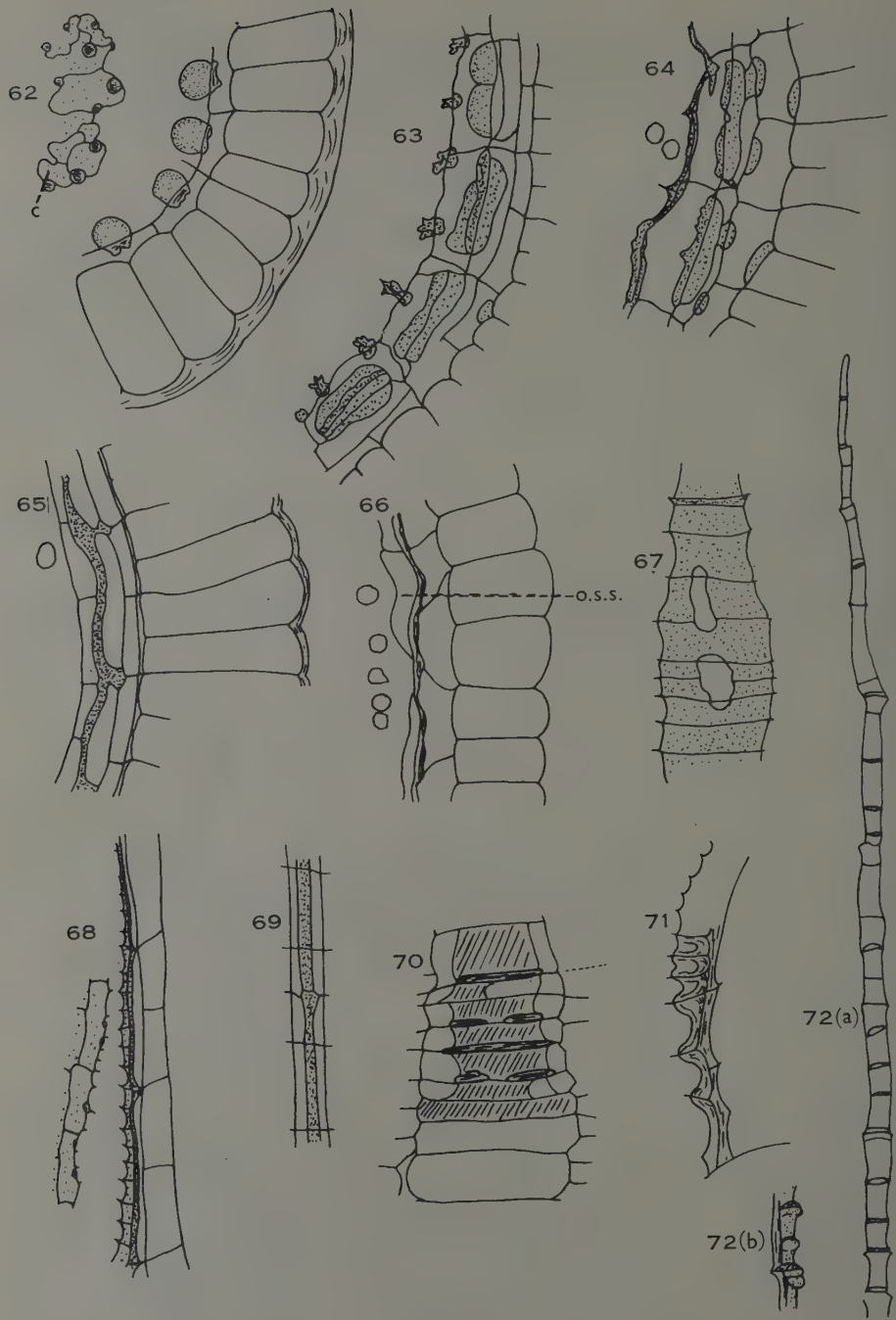
Figs. 49–61 (*Continued*)

of the operculum. Fig. 52.—Cone-shaped region of operculum. Inner peristome developed on periclinal walls between cells of the endothecium and amphithecium. Fig. 53.—Immature outer and inner peristome. The nuclei in the cells of ring 1 are close to the region of deposition of the outer peristome teeth, but strands of cytoplasm pass to the inner peristome processes. Fig. 54.—Similar, in the region of the basal membrane of the inner peristome and the base of the outer peristome teeth. Fig. 55.—Group of three cells which contribute to two peristome teeth. Immature. × *c.* 1000. Fig. 56.—Almost mature outer peristome tooth and developing inner peristome processes. Transverse wall of ring 1 in surface view. × *c.* 1000. Figs. 57–60.—Hand-cut transverse sections of capsules. ×340. Fig. 57.—Outer peristome in tapering region. Endothecial cells with small areas of thickening. Fig. 58.—Outer peristome in cone-shaped region. Anticlinal divisions in cells of ring 1. Inner peristome processes on walls between endothecium and amphithecium. Fig. 59.—Outer peristome in region of rim of capsule. Some thickening on the walls between rings 2 and 3. Basal membrane of inner peristome on walls between endothecium and amphithecium. Fig. 60.—Same capsule as in Fig. 59, at a lower level. Basal membrane of outer peristome. Fig. 61.—Longitudinal section of the upward extension of the columella. ×340.



Figs. 62–66.—Transverse sections of a mature capsule at successively lower levels. Fig. 62.—Outer peristome teeth in tapered region of capsule. Extension of columella (c) with thickened patches. Fig. 63.—Outer and inner peristome in cone-shaped region. Fig. 64.—Region of the rim of the capsule. The outer peristome teeth are attached to

[*For continuation see opposite page*]

of the cells of ring 2 close to the region of deposition. Strands of cytoplasm pass from the nucleus to the regions of deposition furthest away. Figure 45(*b*) shows cells of the peristome layers, near the base of the peristome, just before deposition of thickening. Figures 53–55 show cells with deposition at an early stage. The deposits of cell wall material are at first stained by ruthenium red and by fast green, and are probably largely of cellulose and pectin, but as the peristome matures they become yellow to brown-red and do not stain with fast green.

The thickening on the walls of the cells of ring 2 is denser, but not as thick as that which appears at adjacent positions in the cells of ring 1 (Figs. 57, 62). The deposits of cell wall material in the cells of ring 1 are at first rather ragged at the margins but later are thick and subcylindrical in section with smooth margins (Figs. 55, 56).

Near the apex of the peristome region the deposits in rings 1 and 2 are small and similar in shape to each other, and produce the fine tips of the outer peristome teeth (Figs. 49, 50).

In the cone-shaped region of the operculum the teeth are wider as the deposits in rings 1 and 2 spread further across the dividing periclinal wall (Figs. 58, 63). The deposit is not always even or complete and perforations may occur in the lower part of mature teeth, as seen in tangential view in Figure 67, Plate 1, Figure 3, and Plate 2, Figure 1. A side view of a mature tooth just above the capsule rim (Fig. 71) shows that the deposits from the cells of rings 1 and 2 are practically the same thickness, and ridges on the inner side of a tooth are not as closely spaced in this region as they are for the greater part of the tooth.

Below the rim of the capsule the thickening spreads even further across the dividing periclinal wall until at the base of the outer peristome adjacent teeth are joined to form a continuous basal membrane (Figs. 60, 65). Figures 59, 60, 64, and 65 are transverse sections selected from two series of serial sections, the first of an immature and the second of a mature capsule. Figures 59 and 64 show the wide teeth of the outer peristome just below the rim of the capsule and deposits of thickening on the periclinal wall between rings 2 and 3 may be confluent with the outer peristome teeth. The deposit in ring 1 may have an irregular margin instead of the usual smooth surface. Figure 48 shows a longitudinal section of a mature capsule with thickening on the walls of the cells of ring 3, to which the outer peristome is attached.

Figs. 62–72 (*Continued*)

the capsule wall by extensions of thickening. Basal membrane of inner peristome. Fig. 65.—Basal membrane of outer peristome. Fig. 66.—Spore case region, columella not shown. Outer spore sac against capsule wall. Fig. 67.—Tangential longitudinal view of immature outer peristome tooth just above the rim of the capsule. Fig. 68.—Longitudinal section showing relationship between operculum, outer peristome tooth, and the upward extension of the columella. Fig. 69.—Tangential longitudinal section showing immature outer peristome tooth. Fig. 70.—Similar, showing base of outer peristome tooth. Thickening shaded. Rim of capsule indicated by dotted line. Fig. 71.—Side view of outer peristome tooth just above rim of capsule. Fig. 72(*a*).—Filamentous tip of outer peristome tooth. Fig. 72(*b*).—Lower portion of Fig. 72(*a*) in side view. Figs. 62–72. ×450.

Figures 60 and 65 are in the region of the basal membrane and show extensions of the thickening along the anticlinal walls of ring 2 which firmly attach the outer peristome to ring 3, which in this region forms the inner layer of the capsule wall.

Figure 70 is a tangential longitudinal section of portion of an immature capsule below the rim. It shows the thickening on the periclinal wall (shaded) and the thicker deposits which are sometimes incomplete on the closely spaced transverse walls of ring 2.

Above the rim of the capsule thickening is not deposited on the anticlinal walls or on the junctions of anticlinal and periclinal walls, and when these disintegrate at maturity the peristome teeth are free.

The endothecial tissue inside the peristome consists of large thin-walled cells, but there is an irregular deposition of thickening in small rounded masses on longitudinal and transverse walls and at the junctions of cell walls (Figs. 61, 62, 68), and this region is shed as a rod-like piece of tissue when the operculum is detached. The cells between this rod-like piece and the columella are thin-walled and become flattened before tearing apart when the operculum is shed.

The transverse bars on the dorsal surface of a mature tooth delimit segments which number 14–16 above the broad basal region. In the lower eight segments the trabeculae on the ventral surface are very closely spaced (Plate 2, Figs. 1, 3), then towards the tip the thickening on the inner transverse walls is considerably reduced and the walls are more distant, so that the trabeculae become fewer and further apart until the last segments appear practically smooth (Fig. 72).

No zig-zag line is seen on either dorsal or ventral surface as the teeth are not formed at the junction of anticlinal and periclinal walls as in *Funaria hygrometrica*, *Aulacomnium heterostichum*, and most other members of the Bryales. In side view the remains of the dividing periclinal wall are seen as an irregular longitudinal line, and lines extending to either side are the remnants of the transverse walls of rings 1 and 2 (Fig. 71).

(ii) *Inner Peristome.*—The inner peristome is formed in the lower cone-shaped portion of the opercular region of the capsule and consists of 28–32 (rarely as few as 24 or as many as 36) processes. These processes are produced by the eight cells of ring 1 and the outermost layer of cells of the endothecium, which is the upward extension of the layer which forms the sporogenous tissue in the fertile region of the capsule. This is seen in longitudinal section in Plate 3, Figures 1 and 3, and Plate 4, Figures 1 and 2.

The wall between the two layers which contribute to the inner peristome is the original dividing wall between the embryonic amphithecium and endothecium, and the two layers are respectively the first layer delimited in the amphithecium and the first layer delimited in the endothecium. The inner peristome is formed on this original dividing wall, *P*, in Figure 73, which is a diagrammatic representation of a transverse section of the capsule in the lower cone-shaped region.

In the formation of the processes narrow, somewhat irregular longitudinal strips of cell wall material are first laid down on the inner periclinal walls of the cells of ring 1, adjoining the junctions of the anticlinal walls of the outer endothecial layer

with the dividing periclinal walls. This is shown in transverse section in Figures 52 and 53. At the junctions of the transverse walls with the periclinal walls the deposit is thicker and a slight extension of the deposit occurs to form small plates. Figure 75 is a tangential longitudinal section showing the strips of thickening on the periclinal wall (shaded) between ring 1 and the outer endothecial layer, and the extensions of the deposit on the transverse walls of ring 1.

A deposit of cell wall material contributed by the endothecial cells is later laid down in the angles at the junctions of the anticlinal walls of the endothecial cells with the dividing periclinal wall. This deposit forms a longitudinal angular ridge. Deposits are thicker at the junctions of the transverse walls of the outer endothecial layer with the periclinal walls, as shown in Figure 77.

The deposit of secondary thickening from the outer endothecial cells is much sparser than that contributed by the cells of ring 1 and may sometimes be absent, particularly at the tips of the processes.

The mature processes are somewhat irregular, as the thickening is not uniform and is laid down at the junctions of cell walls (Fig. 80). On disintegration of the walls between the processes they bend inwards to form a dome-shaped structure (Plate 2, Fig. 2).

The inner peristome processes extend from the junction of the operculum with the rim of the capsule to the top of the cone-shaped region of the operculum, and involve 8–10 tiers of cells of ring 1.

Just below the level of the rim of the capsule the periclinal dividing walls receive a thin deposit of cell wall material for a depth of 4–6 cells. These walls form the continuous basal membrane of the inner peristome. Portions of this membrane at various stages of development are shown in Figures 48, 54, 59, 79, 80, and 81. The upper portion of the basal membrane usually receives a thicker deposit of cell wall material and is golden yellow, while the lower part is cream to yellow. In addition there is a hoop-like band of thickening deposited around the upper part of the basal membrane. This is brown, appears to be formed of the same material as the processes, and is deposited at the junction of transverse walls of cells of the outer endothecial layer with the dividing periclinal walls. Figure 64 shows a transverse section of this band of thickening and Figures 76 and 79(*b*) show it in tangential longitudinal view. It is also shown in Plate 1, Figure 3, and Plate 2, Figure 3, which are photographs of the mature peristome from above and below.

Occasionally a second band joins the lower portions of the free processes and anastomoses may occur between the processes at a higher level.

The basal membrane of the inner peristome is loosely attached to the inner wall of the outer spore sac of the fertile region (Plate 4, Figs. 1, 2, and Fig. 48). There is no firm connexion between the outer peristome and the inner peristome at the base and the mature inner peristome may be removed as a unit in the form of a dome as shown in Plate 2, Figure 2.

On the inner side each of the mature processes has an irregular fine line along its length, representing remnants of the anticlinal walls of the outer endothecial layer. The transverse walls of this layer are also represented by fine lines (Figs. 76, 79(*b*)).

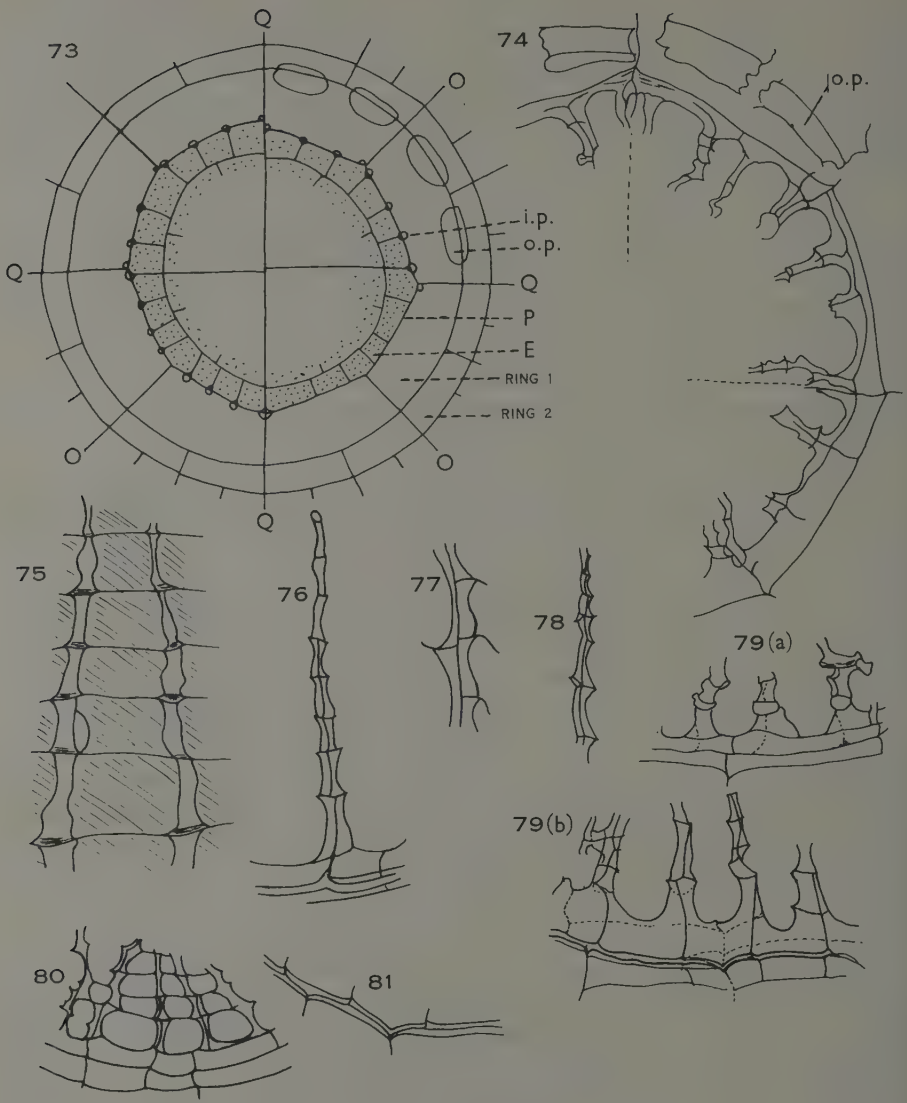


Fig. 73.—Diagram of a transverse section of a capsule of *Mittenia* showing peristome layers, and indicating variations in the development of the inner peristome. *E*, outermost layer of the endothecium; *O*, octant wall of amphithecium; *P*, periclinal wall between ring 1 and the outermost layer of the endothecium; *Q*, quadrant wall. Fig. 74.—Hand-cut section of a mature inner peristome. The dotted lines indicate the position of quadrant walls. Double processes occur at quadrant and octant walls. ×340. Fig. 75.—Tangential longitudinal section of inner peristome developing on the periclinal wall between ring 1 and the endothecium. Dorsal surface. × *c.* 1000. Fig. 76.—Complete inner peristome process with part of basal membrane and hoop-like ring of thickening. Ventral surface. ×450. Fig. 77.—Longitudinal section of immature process, side view. × *c.* 1000. Fig. 78.—Side view of process. ×450. Fig. 79(*a*).—

[*For continuation see opposite page*]

On the outer side of the processes, small plates of thickening are present in the regions where the transverse walls of ring 1 joined the periclinal dividing walls. The longitudinal lines of the inner side are sometimes visible with deeper focusing. Figure 79(*a*) is the outer side of the portion of inner peristome shown in Figure 79(*b*).

In a side view of a process the remnants of the periclinal walls remain as a longitudinal line, and alternating lines extending to either side represent the remnants of the transverse walls of ring 1 and the outer endothecial layer (Figs. 77, 78).

There is a variation in the number of cells of the outer layer of the endothecium which subtends each of the eight cells of ring 1 of the amphithecium, so there is a variation in the number of processes of the inner peristome. There is also a variation in the relationship of the processes to the outer peristome teeth but there is a general approximation to one process opposite and one alternating with each tooth.

At maturity there are usually 24–28 (rarely 32) cells in the outermost endothecial layer, 6–7 (rarely 8) cells subtending two of the amphithecial cells of ring 1. With this arrangement 28–36 processes develop and this is brought about in a manner which will be described with the aid of a diagram shown in Figure 73, as well as drawings of sections of the peristome.

The endothecium develops independently of the amphithecium, but the anticlinal walls of the outer endothecial layer (*E* in Fig. 73) and of ring 1 should coincide at the junctions of the four original quadrants, as here they represent portions of the embryonic walls i and ii shown in Figure 28; these walls are labelled *Q* in Figure 73. At the junctions of the endothecial portions of these four quadrant walls with the original periclinal walls (*P* in Fig. 73) between amphithecium and endothecium, there are three situations which may arise.

(1) Usually the original periclinal walls do not meet exactly at the quadrant walls, and there is some distortion of wall position during growth and slight displacement of the anticlinal quadrant walls occurs so that each one may not coincide exactly in the endothecial layer and ring 1. Actually a small portion of the quadrant wall is pulled into a periclinal position. In these positions, at four points around the circumference, there is commonly a development of a pair of closely spaced processes, one being initiated in each of the two adjoining cells of ring 1. One process develops at the point where the anticlinal wall meets the dividing periclinal wall of one of the cells of ring 1 and another usually smaller process is formed close by, on the inner periclinal wall of the adjoining cell of ring 1. This situation is shown on the left side of Figure 73.

Sometimes the displacement of the quadrant wall is greater and the pair of processes is formed further apart as shown on the right-hand side of Figure 73.

Anastomoses between the processes occur (Plate 2, Fig. 2, and Fig. 58) and are very numerous when the pair are closer together.

Figs. 73–81 (*Continued*)

Dorsal view of part of inner peristome. Fig. 79(*b*).—Ventral view of the same. ×450. Fig. 80.—Tangential longitudinal section of portion of inner peristome. ×340. Fig. 81.—Transverse section of basal membrane of inner peristome. ×450.

(2) If the original periclinal walls do not meet exactly at a quadrant wall and there is no displacement of the quadrant wall, a smaller process may be superimposed on a larger one, as shown at the top quadrant wall in Figure 73. Anastomoses between the pair are then very frequent.

(3) If the periclinal walls meet at the same point on a quadrant wall there is no displacement and one extra large process is formed at the junction as cell wall material is deposited by the two adjoining cells of ring 1 and the two cells of the endothecium. An example of this type is shown on the lower quadrant wall of Figure 73.

In addition a pair of processes is frequently formed at four other points, intermediate between the pairs at the quadrant walls. This occurs when an anticlinal wall of the outer endothecial layer happens to meet the periclinal wall close to its junction with the anticlinal wall (*O* in Fig. 73), separating the two cells of ring 1 in a quadrant. This is usually the case when six cells of the outer endothecial layer subtend two cells of ring 1 as shown in the upper half of Figure 73. This appears to be the most frequent arrangement, so that the processes are arranged in pairs at the junctions of the eight cells of ring 1, with two separate processes alternating with the pairs, producing 32 altogether. Figure 74 is a hand section of a mature peristome of this type, and Plate 2, Figure 2, shows a similar complete inner peristome, with a few irregularities.

In the lower left-hand quadrant in Figure 73, seven cells are shown in the outer endothecial layer, and no double process is formed at the intermediate position if, as in the diagram, an anticlinal wall does not meet the periclinal wall (*P* in Fig. 73) near the dividing wall (*O*) between the two cells of ring 1.

Thus, irregularities in the number of processes occur with variations in the number and the regular development of the outer endothecial cells, and in the presence or absence of double processes. Extra anticlinal divisions which often divide the eight cells of ring 1 (Figs. 53, 58) do not appear to have any effect on the number of processes, which seems to be chiefly related to the number and position of the anticlinal walls in the outer endothecial layer.

(*c*) *Development of the Sporangium*

In the theca the outermost layer of the endothecium forms the archesporium, which is at first a single layer of small cells with large nuclei and densely staining cytoplasm (Figs. 40, 41, and Plate 3, Fig. 1). As seen in transverse section each cell of the original sporogenous layer divides periclinally and anticlinally to produce a square of 16 small isodiametric cells. No further mitotic divisions occur, and these cells form a plasmodium and sporocytes, which undergo meiosis to produce the tetrads of spores. The cell walls do not always disappear before the spore mother cells divide (Plate 4, Fig. 1).

The behaviour of the cells of the inner spore sac and the inner layer of the outer spore sac would suggest that they could be compared with the tapetal cells of higher plants. The cells of these layers adjoining either side of the sporogenous tissue appear to be involved in the nutrition of the developing spores, and become most con-

spicuous with densely staining cytoplasm and large nuclei during tetrad development (Plate 4, Fig. 1). They are crushed and disintegrated as the spores mature (Plate 4, Fig. 2), and finally disappear. The ripe spores are bounded on the inner side by the columella and on the outer side by the remaining layer of the outer spore sac (Fig. 48), which is pushed against the wall of the theca thus obliterating the original air space (Fig. 66).

Lorch (1931) figures a longitudinal section of the fertile region of *Mnium cuspidatum* Leyss. in which there are three layers of cells between the sporogenous tissue and the air space. He prefers to confine the term "outer spore sac" to the innermost of the three layers of cells on the outside of the sporogenous tissue, as this innermost layer is differentiated from the other two in size of cells and arrangement of chloroplasts. Lantzius-Beninga (1850) figures conspicuous chloroplasts in the spore sac cells of several mosses.

In *Mittenia* chloroplasts are present in the capsule wall, in the outer layer of the outer spore sac, and in the columella. A few smaller chloroplasts are present in the inner layer of the outer spore sac and in the inner spore sac, but are not a conspicuous feature.

Starch grains in the columella, the outer layer of the outer spore sac, and the exothecium become particularly conspicuous as the spores mature (Plate 4, Fig. 2).

V. Discussion

The capsule of *Mittenia* has previously been described as having no stomata, but a few are usually present on the lower portion of the theca.

Muller (1901), Rodway (1914), and Sainsbury (1955) describe *Mittenia* as having 16 outer peristome teeth and 16 inner short filiform nodular processes from a low basal membrane. Mitten (1860) in the original Latin description describes the peristome: "Peristomium externum dentibus 16, internum processibus alternatibus, in membrana exsertis". The capsules of the moss from the ranges near Melbourne had 28–32 inner processes.

The peristome is of primary importance in the classification of mosses but there are few complete descriptions of the development of the peristome in the Bryales.

Blomquist and Robertson (1941) reviewed the literature on the moss peristome and remarked on the scarcity of accounts which included the embryonal development. They gave a detailed account of the development of the double peristome of *Aulacomnium heterostichum* (Hedw.) B. and S., which is derived from the three innermost layers of the amphithecium, no deposition occurring on the periclinal walls between amphithecial and endothecial cells, and suggested that this is the usual method of development in members of the Diplolepideae.

Proskauer (1958) investigated the peristome of *Funaria hygrometrica* and clarified previous conflicting accounts of its development. The double peristome is derived from the three innermost layers of the amphithecium, no deposition occurring on the periclinal walls between amphithecium and endothecium.

The figure of the longitudinal section of a mature peristome of *Hypnum sylvaticum* in the article by Lantzius-Beninga (1850) shows the deposition of thickening which forms the inner peristome apparently on the inner periclinal walls of the innermost layer of the amphithecium (that is, on the walls separating amphithecial and endothecial cells) but no thickening on the endothecial side of the walls. van der Wijk (1932) interpreted this figure in a similar manner but Blomquist and Robertson considered it to be a misinterpretation because of the similarity of the mature peristome of *Aulacomnium heterostichum* to the peristome in the figure of *H. sylvaticum*.

As mentioned by Proskauer (1958) and Blomquist and Robertson (1941), Lorch (1931) when discussing *Mnium cuspidatum* suggested that the inner peristome layer is endothecial in origin, but no previous account which includes early stages of development has shown that the walls which form the inner peristome are between amphithecium and endothecium.

The only single peristomate moss studied in detail is *Ceratodon purpureus* (Hedw.) Brid. (Evans and Hooker 1913). Philibert (1888, paper not seen) considered the single peristome in the Haplolepideae to be homologous with the inner peristome in the Diplolepideae. In *C. purpureus* the thickening develops on both sides of the walls between the cells of the two innermost layers of the amphithecium. This would correspond to the position of the inner peristome in *F. hygrometrica* and *A. heterostichum* as suggested by Evans and Hooker (1913) and Blomquist and Robertson (1941), but it would equally well correspond to the position of the outer peristome of *Mittenia*.

Support for this latter statement is perhaps to be derived from the following observations. In *A. heterostichum*, *F. hygrometrica*, and *Mittenia*, three layers contribute to the double peristome. In *Mittenia* the middle layer (ring 1) and the outer layer (ring 2) are derived from the amphithecium, the inner peristome layer from the endothecium. In *A. heterostichum* and *F. hygrometrica* the three peristome layers are the three inner amphithecial layers. In each of the three mosses the second layer of the amphithecium outside the endothecium (that is, the middle peristome layer of *A. heterostichum* and *F. hygrometrica* and the outer peristome layer of *Mittenia*) is composed of 16 cells. Lantzius-Beninga figures sections of mature capsules (with no developmental stages) of several members of the Haplolepideae and Diplolepideae which also have this layer of 16 cells in common. The number of cells corresponds to the usual number of outer peristome teeth of a double peristomate moss and the usual number of teeth of a moss with a single peristome. The outer peristome of the double peristomate mosses like *F. hygrometrica* and *A. heterostichum* develops from this layer and the next layer outside. In *Mittenia*, however, the outer peristome develops from this layer of 16 cells and the next layer inside, as is also the case in the single peristomate *C. purpureus*, *Didymodon rubellus* (Hoffm.) Bryoleur., figured by Lantzius-Beninga under the name *Weissia recurvirostra*, and *Pottia bryoides* (syn. *Mildeela*), figured by Limpricht (1890).

A comparison may be made of the numbers of cells in each of the peristome layers (as seen in transverse section) of the various mosses which have been investigated.

Mittenia has a small and relatively simple capsule and there are not six concentric layers in the amphithecium, as in most mosses which have been fully described, but three in the greater part of the peristome region, four at the base. *Pottia bryoides* and *D. rubellus* mentioned above also appear to have 3–4 layers of amphithecial tissue in the peristome region, the two innermost being peristome layers as in *C. purpureus*. Limpricht's drawing would indicate that *P. bryoides* has only 16 cells in each of the peristome layers. *D. rubellus* according to Lantzius-Beninga's figure has 32 in the inner layer, 16 in the outer layer. In *C. purpureus* the inner layer has 24 cells (eight large cells each divided into three by anticlinal walls) and the outer layer 16 cells.

In *Mittenia* the innermost layer of the amphithecium consists of eight large cells and contributes most of the peristome thickening while the outer peristome layer has 16 cells. The outer layer of the endothecium has 24–28 cells and contributes to the inner peristome.

In *F. hygrometrica* and *A. heterostichum* the middle peristome layer consists of 16 cells, the outer layer of 32 cells. The inner layer of amphithecium in *F. hygrometrica* is composed of 32 cells, in *A. heterostichum* of 64–72 cells, and in both these mosses this layer contributes to the inner peristome on its outer periclinal wall. The variation in numbers in the inner peristome layers in *A. heterostichum* and *Mittenia* causes a variation in the numbers of inner processes in mature capsules in each case.

The embryonal development shows that the inner peristome of *Mittenia* develops on the walls separating amphithecium from endothecium, with material contributed by both of the primary regions, and in this *Mittenia* differs from other members of the Bryales in which investigation has included the early stages of development.

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EXPLANATION OF PLATES 1–4

PLATE 1

Fig. 1.—Portion of the gametophore of *Mittenia plumula* showing leaves and stem. × *c.* 100.

Fig. 2.—Capsule of *Mittenia*. × *c.* 40.

Fig. 3.—Detached peristome and rim of capsule from above. The teeth of the outer peristome have curled outwards and the trabeculae on the ventral surface can be seen. No restriction being imposed by the capsule wall, some of the tips of the teeth have curled under and come up between the inner peristome processes. The hoop of thickening can be seen around the basal membrane of the inner peristome, and there are spores between the processes. × *c.* 140.

PLATE 2

Fig. 1.—Side view of portion of the theca wall, rim of capsule, and peristome. The ventral lamellae and the ridges on the dorsal surfaces which mark the articulations where the teeth bend are visible. The relationship of the inner peristome to the outer may be seen. × *c.* 100.

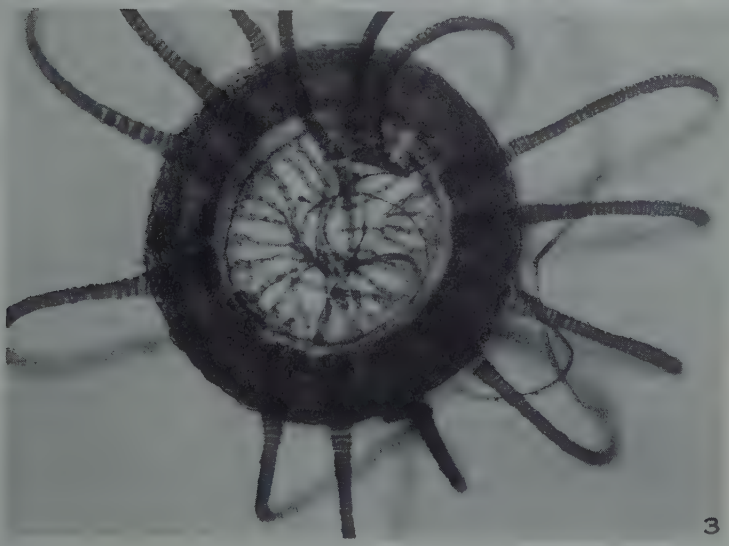
Fig. 2.—Inner peristome removed from the capsule. There are approximately 32 processes. Two single processes alternating with pairs of processes, which is the usual arrangement, are seen on the right. × *c.* 250.

Fig. 3.—Similar to Plate 1, Figure 3, but photographed from below. There is an abnormal bifurcation in one of the outer peristome teeth. × *c.* 100.

PLATE 3

Longitudinal sections of young capsules showing the layers of cells which contribute to the formation of the outer and inner peristome (cf. Fig. 47). × *c.* 390.

GAMETOPHORE AND SPOROPHYTE OF MITTENIA PLUMULA



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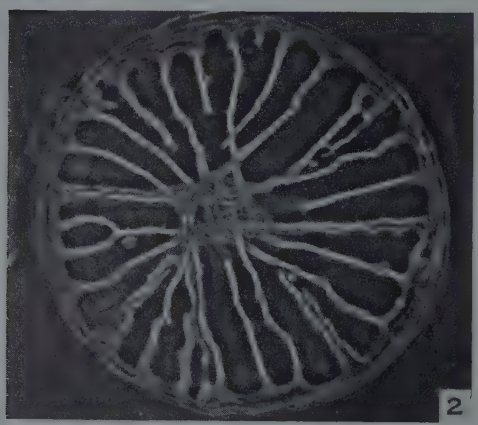

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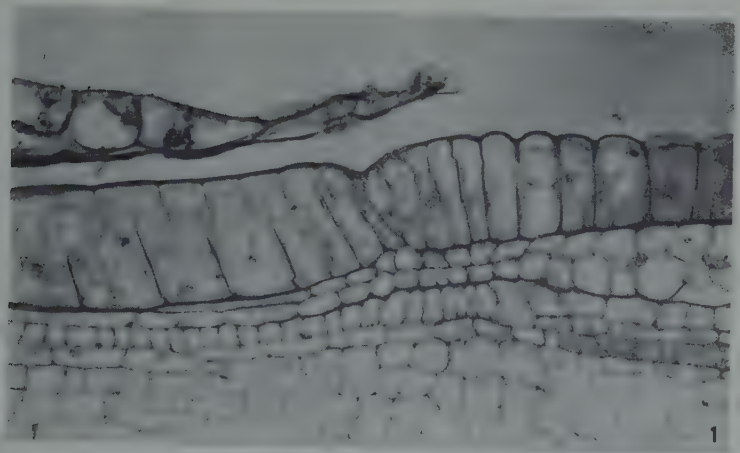

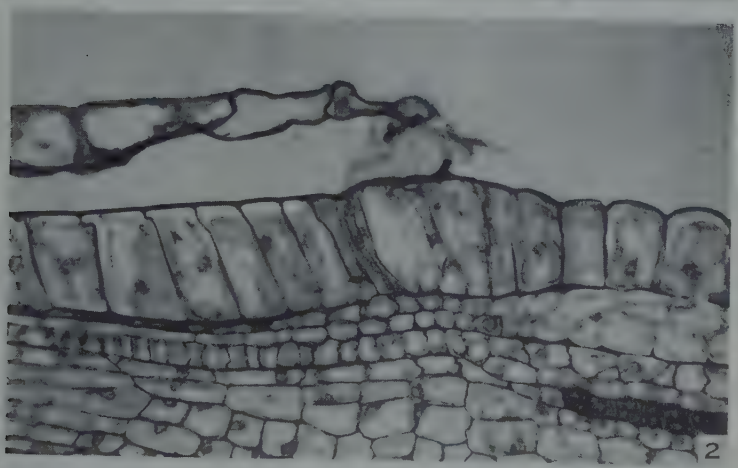

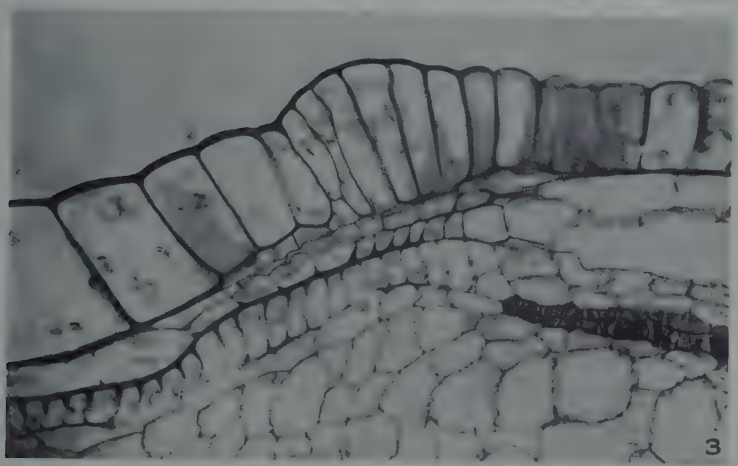

GAMETOPHORE AND SPOROPHYTE OF MITTENIA PLUMULA

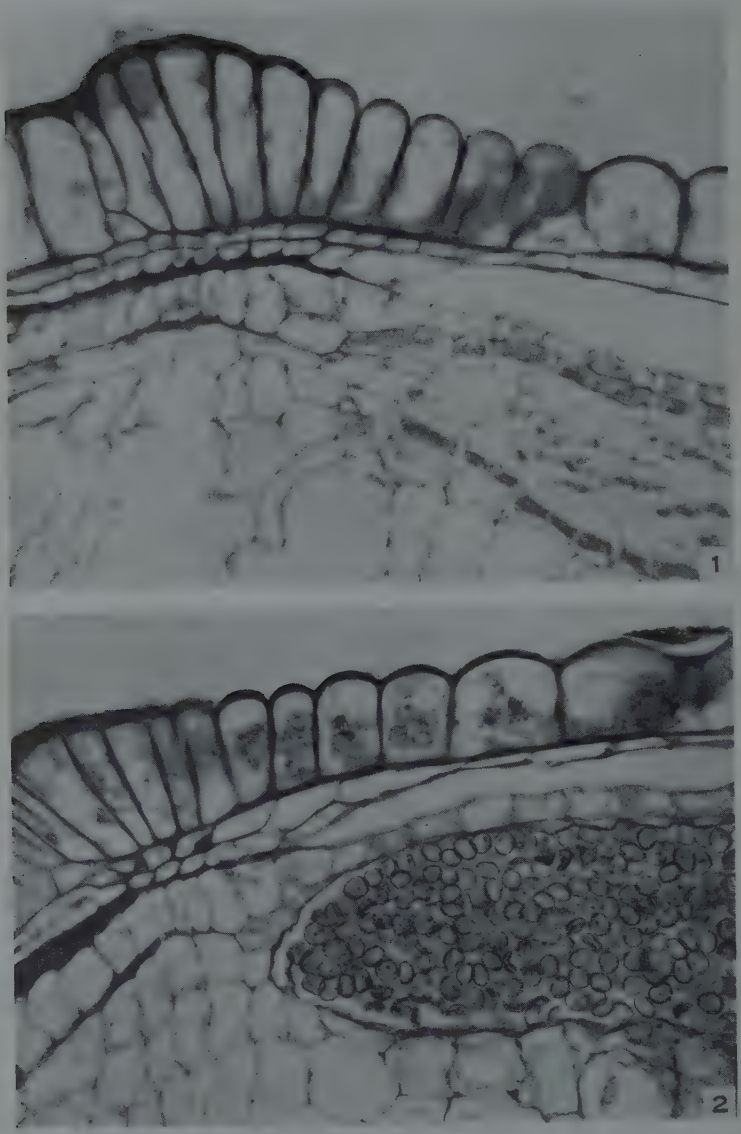

Fig. 1.—Note rings 1, 2, and 3 of the amphithecium. Ring 1 has divided into two layers (the outer spore sac) in the fertile region, below the rim of the capsule. The deeply stained layer of the archesporium and its upward extension is the outermost layer of the endothecium. There is a very small amount of thickening on the wall between endothecium and amphithecium in the region of the basal membrane of the inner peristome, also on the wall separating rings 1 and 2 where the outer peristome and its basal membrane develop. There is a defined line between the outer spore sac and the cells of ring 2 where the air space seen at the base will develop.

Fig. 2.—Similar to Figure 1, but the archesporial tissue is two-layered.

Fig. 3.—Somewhat older capsule, with thickening of inner and outer peristomes more defined. Note air space between the outer spore sac and the capsule wall.

Plate 4

Similar to Plate 3, but older capsules. Basal membranes and lower parts of inner and outer peristomes are well developed. × *c.* 390.

Fig. 1.—Note development of archesporial tissue and deeply stained contents of cells of inner spore sac and inner layer of outer spore sac.

Fig. 2.—The spores are almost mature and the air space diminished in size. There is some plasmodium still present, and the cells of the inner spore sac and inner layer of the outer spore sac are crushed.

STUDIES ON THE ORIGIN, EVOLUTION, AND DISTRIBUTION OF THE GRAMINEAE

IV. THE GENUS POA L.

By W. HARTLEY*

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Summary

Studies of the regional and global distribution of the genus *Poa* L. show that it reaches its highest relative specific differentiation in regions of high latitude and high altitude. The genus is absent or rare in the tropics, except in mountainous regions, but constitutes more than 15% of the grass flora of Alaska, Iceland, Kamchatka, and the high Pamir Mountains.

There is a close association between the occurrence of high percentage frequencies of species of *Poa* and cool summer temperatures. In the United States the 75°F mid-summer (July) isotherm effectively demarcates those regions in which *Poa* spp. form more than 5% of the grass flora from those in which they constitute a smaller percentage. A similar relationship between percentage frequency and mid-summer temperature is found in most other parts of the world. Climatic factors other than temperature seem to have little influence on the distribution of *Poa*.

It is concluded that the genus has attained a high stage of development in nearly all parts of the world to which it is climatically adapted. The evidence does not permit firm conclusions about the centre of origin of the genus, but it has a major centre of species differentiation in the mountain and plateau region of central Asia. It is probably monophyletic, and has had a long evolutionary history in the course of which it has spread through regions now separated by topographical or climatic barriers.

I. INTRODUCTION

Previous papers in this series (Hartley 1958*a*, 1958*b*; Hartley and Slater 1960) have dealt with the distribution of the grass tribes Andropogoneae and Paniceae, and the tribes of the subfamily Eragrostoideae. All these taxa are cosmopolitan in distribution, but with a main concentration in the tropical and subtropical parts of the world.

In the present paper, the temperate genus *Poa* L. is reviewed, using, as the basis for distribution mapping, the percentage frequency of species of the genus in comparison with the total number of grass species occurring in each region.

II. THE GENUS POA L.

Poa is one of the larger genera of Gramineae, with more than 200 species. It is thus comparable in size with some of the smaller tribes discussed in previous papers. Taxonomically the genus is relatively homogeneous and well defined, although it was formerly taken to include many species which are now placed in unrelated genera such as *Eragrostis*. There is, however, less agreement among taxonomists regarding the subdivision of the genus into natural groups or sections.

* Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T.

Thus the North American species have been treated differently by Hitchcock (1951), Marsh (1952), and Clausen and Hiesey (1958). Further, the very frequent occurrence of apomixis in the genus has resulted in differing viewpoints about the limits of individual species.

Poa is one of the most widely distributed of grass genera. It is included by Good (1953) among the 21 genera of grasses with a cosmopolitan or subcosmopolitan distribution, being listed with *Bromus*, *Deschampsia*, and *Hierochloe* among the genera "which, although essentially temperate in character, have nevertheless a notable distribution through the more elevated parts of the tropics". The relationship between distribution and temperate climate is also stressed by Clausen and Hiesey (1958): "The Poas are for the most part species of cool or cold environments. They cluster at high latitudes in both the Northern and Southern Hemispheres, and at lower latitudes they occur mostly on the higher mountain ranges".

Within the broad limits of the distribution of the genus, species of *Poa* occupy very diversified habitats. Bews (1929) lists among the typical habitats occupied by *Poa* woodlands, marshes and other hygrophilous situations, moist meadows, meadows and pastures, sand dunes, and alpine and ruderal situations, while species occur frequently in arid and semi-arid habitats. Several of the more widespread species, such as *Poa pratensis* L., have developed numerous ecotypes which enable the species to occupy many different habitats.

From this brief survey, it seems that the genus *Poa* has some of the characteristics exhibited by the tribes of grasses discussed in previous papers in this series, viz.:

(1) A degree of taxonomic homogeneity which suggests monophyletic origin.

(2) Cosmopolitan or subcosmopolitan distribution.

(3) An apparent relationship between distribution and climatic factors.

(4) A wide range of ecological amplitude.

It is thus of interest to examine the distribution pattern more closely because of the light which this may shed on the evolutionary history and status of the genus.

(*a*) *The Distribution of* Poa

The distribution of *Poa* may be mapped conveniently, both on a regional and world basis, by the percentage frequency method used in previous papers in this series. In this method, the relative importance of the genus in the grass flora of any region is shown as the number of species of *Poa* present, expressed as a percentage of the total number of grass species.

In Figure 1, the distribution of *Poa* in the British Isles is shown on this basis. The map is based on data given by Druce (1932), and indicates those vice-counties in which *Poa* spp. form respectively more or less than 10% of the grass flora. It will be noted that in all the vice-counties of Ireland, as well as those of the lowland areas of England, Wales, and southern Scotland, less than 10% of the grasses are species of *Poa*. By contrast, in the highlands of Scotland and in some of the more mountainous vice-counties of Wales and northern England, more than 10% of the grasses are species of *Poa*.

In the British Isles the effects of latitude and altitude are not sharply distinguished, as the more northerly regions are, in general, more mountainous. The effect of latitude, relatively uninfluenced by altitudinal differences, can be more clearly shown by data on grass distribution in the Scandinavian peninsula. Table 1, based on the distribution maps published by Hulten (1950), shows the relationship

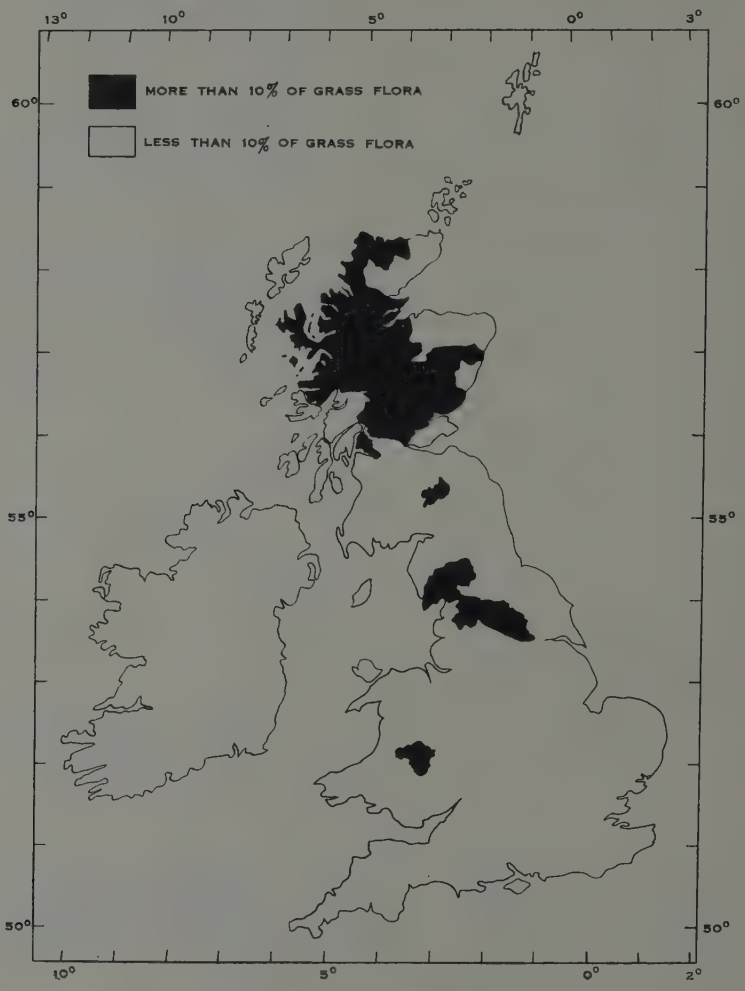

Fig. 1.—Distribution of *Poa* in the British Isles.

between the percentage frequency of *Poa* and latitude in Scandinavia. This table shows clearly the progressive and fairly regular increase in the percentage frequency of species of *Poa* in the grass flora in successive latitudinal zones from the southern limits of Scandinavia to the $67\frac{1}{2}$–$72\frac{1}{2}°$ zone in the far north.

It is not readily possible to show in a similar way the effect of altitude uncomplicated by latitude. It is, however, well known that, especially in tropical and

subtropical regions, species of *Poa* occur most frequently at high altitudes. Thus, only three species of *Poa* are recorded from Kenya (Bogdan 1958), and these are restricted to altitudes above 6500 ft.

Figure 2 shows the world distribution of *Poa* based on data collated from more than 350 floras and floristic lists. Each symbol on the map represents the approximate geographic centre of a region covered by one of these lists. Different symbols are used to indicate the levels of percentage frequency of species of *Poa* in the regions concerned. In addition, lines have been drawn to show the 5% and 10% frequency limits. The few symbols which fall outside the frequency zones as indicated by these lines are circled.

TABLE 1
DISTRIBUTION OF POA IN RELATION TO LATITUDE IN SCANDINAVIA
(DATA FROM HULTEN 1950)

Latitudinal Zone	Number of Species		*Poa* Spp. as Percentage of Grass Flora
	Poa	Total Grasses	
55 –57½°N.	13	116	11·2
57½–60°N.	16	127	12·6
60 –62½°N.	17	125	13·6
62½–65°N.	16	110	14·5
65 –67½°N.	14	94	14·9
67½–72½°N.	13	72	18·1

The map reveals several features of interest:

(1) The distribution pattern is regular and consistent throughout the world, with few "anomalous" frequency values.

(2) Low frequencies of *Poa* are found throughout the inter-tropical zone, with the exception of the high Andes Mountains. In general, the genus is absent from tropical lowlands.

(3) The 5% frequency line runs approximately along the 40° parallel of latitude in the Northern Hemisphere, with deviations to the south in regions of high altitude (Rocky Mountains, Appalachian Mountains, Caucasus Mountains, Pamirs, and Himalayas), and to the north in regions of flat plain (coastal eastern U.S.A., Trans-Caspian region).

(4) In the Southern Hemisphere the 5% frequency line is less well defined, partly because of the inadequacy of the floristic data, but corresponds approximately to the 35°S. parallel except in the Andean region of South America.

(5) The few higher percentages within the "under 5%" zone refer to high mountain regions (Atlas Mountains, Djebel Druze Mountains). A minor exception to this is the State of Indiana, U.S.A., which has a percentage frequency of 5·3% and is shown just south of the 5% line on the map.

(6) The 10% frequency line does not follow closely any particular parallel of latitude, but deviates towards the Equator in mountainous regions (Rocky Mountains, Andes Mountains, Mongolia) and towards the poles in regions of extensive plains (eastern United States and Canada, eastern Europe, and western Asia). The few higher values shown south of this line in the Northern Hemisphere are usually in mountain regions (Pamirs, Switzerland, Czechoslovakia, northern Spain).

(7) The highest percentage frequencies of *Poa* (above 15%) all occur in regions of high latitude (Alaska, Iceland, eastern Fennoscania, Kamchatka), or in regions of very high elevation (Pamir Mountains).*

(8) Except in so far as these may be attributed to the effects of regional topography, there are no apparent differences in the distribution pattern between the Eastern and Western Hemispheres.

Thus the global distribution pattern supports the evidence from regional studies that the genus *Poa* shows the highest degree of relative specific differentiation in regions of high latitude or high altitude.

(*b*) *Climate and Distribution*

The occurrence of high percentage frequencies of species of *Poa* in regions of high latitude or high altitude suggests strongly that temperature has a predominant influence on distribution. Indeed, examination of both regional and world distribution maps reveals little if any evidence of the effect of climatic factors other than temperature. Within any particular temperature zone, species of *Poa* occur with approximately the same percentage frequency in regions of high and low rainfall. This is particularly clear in the tropics, the genus being completely absent or occurring in very low frequency both in deserts and regions of tropical forest. The same feature is also evident in the more temperate regions, although in mountainous areas high altitude is normally associated with high rainfall. Where this normal association is reversed, as in the cold dry elevated regions of Tadzhikistan and Mongolia, the occurrence of high percentages of *Poa* indicates clearly that temperature rather than rainfall is the predominant factor in distribution.

Similarly, the increase in percentage frequency with increasing altitude in both the tropical and temperate zones shows that length of day, acting through the photoperiodic responses of the plants, does not have a major influence on the broad distribution pattern, although it is undoubtedly important in the distribution of individual species, and especially of infra-specific taxa.

Comparison of the distribution maps with regional and world temperature maps indicates that the distribution of *Poa* is related to summer temperatures, rather than to winter or annual temperatures. This is particularly evident in the United States, where the percentage frequency isograms follow closely in trend the

* These regions are all in the Northern Hemisphere. However, similar high values occur in the higher latitudes of the Southern Hemisphere. A rather old and probably incomplete list of plants occurring in the Southland District, in the extreme south of New Zealand, records 15 species of *Poa* in a total of 69 grass species (Smith 1914). This gives a very high percentage frequency (21·7%), but is not shown on the map because the total number of grass species is below the minimum level (70 spp.) accepted as the basis in the selection of floras used for mapping.

Fig. 2.—World distribution of the genus *Poa* (as percentage of the species of *Poa* in the total grass flora).

summer (June–August) isotherms as shown in the "Atlas of American Agriculture" (U.S. Department of Agriculture 1936). The relationship to summer temperature is also demonstrated by the data in Table 2, shown graphically in Figure 3.

In Table 2, mean July temperatures are shown for each of the American States, these being arithmetic means of the figures for selected localities in each State given in the "Atlas of American Agriculture". The percentage of species of *Poa* in the grass flora of each State has been calculated from the distribution data given by Hitchcock (1951). The smaller States of New England have been grouped together, as the grass distribution data are inadequate for separate treatment.

The close inverse relationship between mean July temperature and percentage frequency of *Poa* is clearly evident from Figure 3, in which the same data are shown diagrammatically. In the lower part of the diagram the only point which deviates from the general pattern is that representing Maine, which has a lower percentage of species of *Poa* than would be expected from the July temperature. There is a greater scattering of points in the upper part of the diagram, representing mainly the north-western States, but this is readily explained by the great variations in topography in this region. It is probable that the method used for assessing State mean monthly temperatures gives insufficient weight to the areas at the higher altitudes in which species of *Poa* are particularly frequent.

The lines superimposed on Figure 3 show that, with few and minor exceptions, the 75°F July isotherm effectively separates those States with respectively more and less than 5% of species of *Poa* in the grass flora.

It is of interest to consider whether a similar relationship between mid-summer temperature and the 5% frequency line can be demonstrated in other parts of the world. Unfortunately the inadequacies of temperature data, as well as the lack of reliable floras for many parts of the world, preclude a detailed examination of this problem. However, judging from available temperature maps and allowing for the gaps in the floristic records, there is a very good agreement between the 75°F July isotherm and the 5% frequency limit throughout the Northern Hemisphere. The lines coincide closely in the Mediterranean region and in those parts of central Asia where the floristic data provide adequate definition, but the lack of information about grass distribution in China and adjacent regions does not permit comparison in the Far East.

The gaps in the floristic coverage are even more evident in the Southern Hemisphere, but within the limits of the available data there is a good agreement between the 75°F mid-summer (January) isotherm and the 5% frequency line in South America. However, in southern Africa there appear to be no regions in which species of *Poa* constitute more than 5% of the grasses. The south-western and southern fringes of the continent have mean January temperatures below 75°F, and comparable low temperatures occur in elevated regions in the eastern Cape Province, Basutoland, and the Orange Free State. In South Africa, therefore, species of *Poa* form a smaller percentage of the grass flora than would be expected from the summer temperatures.

In the Australian region, the areas in which the percentage frequency of *Poa* exceeds 5% (Fig. 2) include the southern parts of South Australia, most of Victoria,

and all of Tasmania and New Zealand, but exclude Western Australia and New South Wales. Unfortunately, the data on distribution given in most of the Australian floras are scanty and incomplete, and it would be unrealistic to place too much reliance on the map in this region. It is, however, noteworthy that, while the 5% line agrees well with the 75°F January isotherm in South Australia and Victoria, it fails to include the south-western part of Western Australia, and especially the

TABLE 2

MID-SUMMER TEMPERATURE AND DISTRIBUTION OF POA IN THE UNITED STATES

State	Mean July Temperature (°F)	Percentage of Spp. of *Poa*	State	Mean July Temperature (°F)	Percentage of Spp. of *Poa*
Alabama	80·1	2·8	Nevada	72·4	11·2
Arizona	82·3	3·4	New Jersey	74·8	3·7
Arkansas	80·8	3·1	New Mexico	73·3	6·2
California	74·4	8·7	New York	69·9	6·3
Colorado	69·7	11·0	North Carolina	77·7	3·4
Florida	81·3	1·1	North Dakota	67·7	9·9
Georgia	80·6	2·3	Ohio	73·9	6·0
Idaho	69·6	14·3	Oklahoma	81·6	3·8
Illinois	75·8	4·9	Oregon	66·3	12·0
Indiana	75·7	5·3	Pennsylvania	73·2	5·5
Iowa	74·0	5·1	South Carolina	80·4	3·1
Kansas	78·2	3·4	South Dakota	72·2	8·4
Kentucky	76·9	5·8	Tennessee	77·6	4·0
Louisiana	81·8	1·7	Texas	82·6	1·7
Maine	64·9	5·1	Utah	73·1	12·2
Maryland	76·1	5·0	Virginia	76·3	3·6
Michigan	67·9	6·7	Washington	64·8	12·5
Minnesota	69·1	9·7	West Virginia	73·0	5·8
Mississippi	80·5	2·6	Wisconsin	70·2	6·9
Missouri	76·9	3·1	Wyoming	66·1	14·3
Montana	67·7	15·4	New England (excl. Maine)	69·5	4·8
Nebraska	73·7	9·3			

Northern and Southern Tablelands regions of New South Wales, all of which have summer temperatures below 75°F. In these regions the percentage frequency of *Poa* is less than expected from the summer temperatures.

An explanation of the apparently anomalous distribution pattern of *Poa* in south-eastern Australia may be found in the inadequacy of the current taxonomic treatment of the genus. In most Australian floras, many very different forms are all referred to the single species *Poa caespitosa* Forst. Comparable complexes elsewhere have been split into a number of separate species and it is anticipated that current taxonomic studies will result in the recognition of several new species of *Poa* in south-eastern Australia (Joyce W. Vickery, personal communication). Thus some, at least, of the apparent anomalies may be removed.

It is concluded from this survey that the distribution of *Poa* throughout the world, as expressed on the percentage frequency basis used, shows a close relationship with summer temperature, and especially with the temperature of the mid-summer month. This relationship is consistent throughout the Northern Hemisphere and, with the exception of parts of South Africa and possibly south-eastern Australia, also in the Southern Hemisphere.

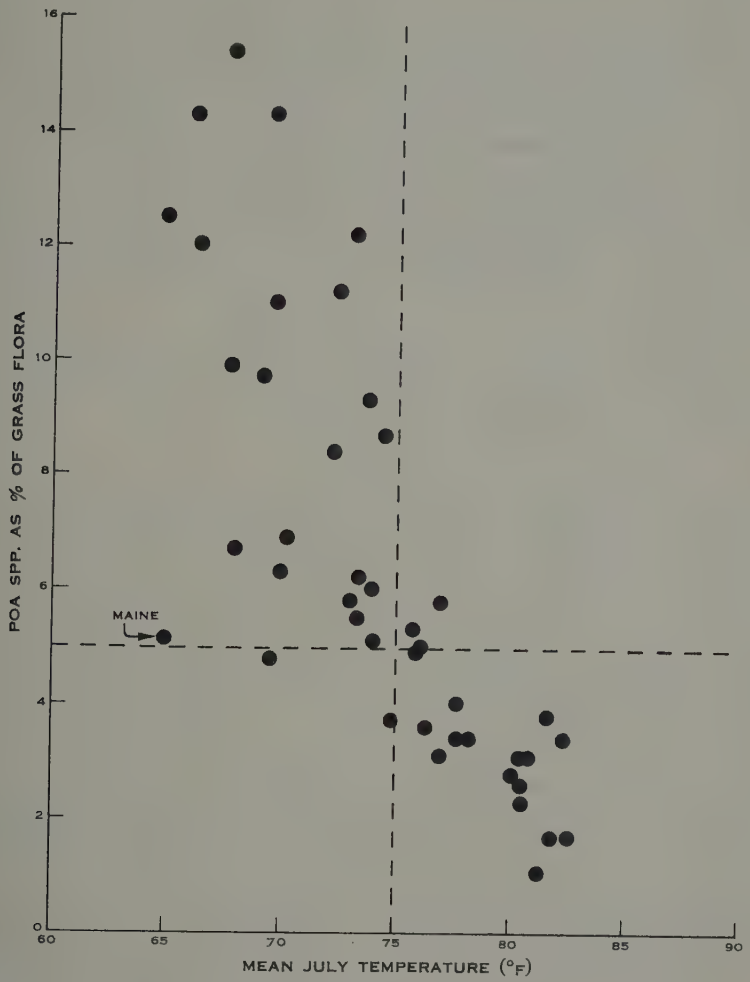

Fig. 3.—Percentage frequency of *Poa* in relation to July temperatures in the States of the U.S.A.

The physiological basis of this relationship cannot be deduced from a survey of this kind and it is evident that the climatic factors involved are more complex than is implied by the use of the mean temperature of the mid-summer month as the sole criterion. However, it may be noted that Hiesey (1953) found that in most of the ecologic races of five species of *Poa* which were studied under controlled temperatures, vegetative growth and flowering were favoured by low temperatures and

particularly by low night temperatures. If this is the normal pattern in the genus, it would help to explain the competitive advantage of species of *Poa* when growing in regions of cool summer.

(c) *Geographical Distribution and Evolutionary History*

As has been pointed out in discussing the world distribution of the genus, the localities with the highest percentage frequencies of species of *Poa* are scattered throughout the Northern Hemisphere in regions of high latitude or high altitude. There is no single region which stands out on the map as a possible centre in which the genus may have originated and from which it has spread. There is, however, evidence of the existence of a major centre of species differentiation in the mountainous region extending from northern Pakistan, India, and Burma to the southern U.S.S.R. and Mongolia. Species of *Poa* are specially numerous, not only in the Pamirs, but also in the Himalayan region. Bor (1960) lists 52 species of *Poa* in India, Burma, and Pakistan, nearly all of which occur in the Himalayas. This is about 25% of the known species of the genus. However, it is not possible, in the absence of a comprehensive monograph of the genus throughout the world, to speculate usefully on whether this region is indeed the centre of origin of *Poa* or a secondary centre with a favourably cold climate in which species differentiation has been prolific.

The most striking feature of the distribution map is the very wide range of the genus, and the close relationship between the distribution and the present climates of the regions in which it occurs. This relationship exists not only between the Eastern and Western Hemispheres, but also, if to a smaller degree, between the Northern and Southern Hemispheres. It is explicable only on the assumption that the genus is a very old one, which has evolved and spread during a long period when some of the present barriers to distribution—topographical and climatic—did not exist. The comparable level of development in the temperate regions of the Northern and Southern Hemispheres is specially significant. If it be postulated that the genus originated somewhere in the higher latitudes of the Northern Hemisphere, it would be necessary to assume that it spread southwards through the tropics, where the climates are at present quite unfavourable to its development, and subsequently developed a high degree of species differentiation in widely separated regions in the high latitudes of the Southern Hemisphere. Similar difficulties would be encountered in any postulated origin in the Southern Hemisphere. An origin in mountainous regions at lower latitudes, or in the tropics at a period when the climate was much cooler there than at present, would help to explain the present wide distribution, but it would still be necessary to assume a long period of development and spread.

The picture of evolutionary maturity presented here, based mainly on the very wide distribution of the genus and the close relationship between the distribution pattern and present climates in all parts of the world, may appear inconsistent with evidence from other sources about the genetic and cytological complexity of *Poa* and the great diversity of reproductive mechanisms which exist within it. Polyploidy and aneuploidy are particularly common in *Poa*, and are often associated with a high degree of apomixis. There are several dioecious species—rare elsewhere among the

grasses—while there are both inter- and intra-specific differences in cross-fertility and self-fertility. In some species, such as *Poa bulbosa* L., the development of bulbils frequently replaces that of seeds in the inflorescence. There is also great physiological and morphological diversity within the genus.

It might be supposed that these great differences indicate that the genus is in a stage of active evolution which would enable it to spread into regions which it does not at present occupy. However, the taxonomic and geographic evidence strongly suggests that *Poa* is a monophyletic genus which throughout its evolution has maintained a consistent pattern of physiological response which enables the species of the genus to develop well in regions with cool summers. It has been shown that the genus is well developed in such regions in almost all parts of the world. On this view, the potentialities for further extension would be very limited, and the genetic and cytological complexities have significance only for micro-evolution and in enabling the species of *Poa* to occupy more completely the range of ecological niches which exist within its overall limits of climatic tolerance.

III. Acknowledgments

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OBJECTIVE METHODS FOR THE CLASSIFICATION OF VEGETATION

IV. PATTERN AND MINIMAL AREA

By D. W. Goodall*

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Summary

The structure of salt marshes and mallee scrub in Victoria and rain forest in Uganda was studied by means of quadrats forming a continuous grid or transect or distributed by restricted randomization; the measures used were cover or basal area. The course of increase in variance as the mean spacing between the sample areas increased was in most cases found to be continuous, a linear regression between the logarithms of the variables fitting the data in many cases. In other instances a quadratic or higher-order regression was indicated, and some of these approximated to a "stepwise" curve of the type suggested by Greig-Smith and others. This appeared, however, to be the exception. The theoretical implications of a continuous increase of variance with spacing are briefly discussed.

I. Introduction

When vegetation is studied by sampling, observations on replicate samples inevitably differ from one to another; and before the investigator can use the sample observations as a basis for describing the vegetation he is obliged to consider the extent to which his samples are representative of the vegetation as a whole, and what meaning to attach to the differences he has observed among his replicates.

The question then arises: What is the smallest sample large enough to show within its boundaries all the features of the vegetation of interest to the ecologist? How large does a sample need to be in order that one should be able to reconstruct from the sample data the whole of the significant pattern in the vegetation? What is the size of the unit cell of which the vegetation is composed—the unit whose repetitions make up the characteristic mosaic pattern, if one exists? This unit area which is large enough to show within itself all the characteristics of the community—at least when sufficiently replicated—has sometimes been called its "minimal area", "representative area", or "Entfaltungsfläche" (Ramenskij 1932).

This question of minimal or representative area has been approached in the past from two main positions. Either the aim has been to ensure that each sample area contains an adequate number of species (i.e. the minimal area has been based on the species–area curve, cf. Cain 1938, Vestal 1949, Hopkins 1955, etc.); or a satisfactory measure of agreement (in respect of each species) between replicate samples has been sought. A discussion of these different approaches will be found in an earlier review paper (Goodall 1952). The second seems likely to be more helpful when one is interested in *which* species are present and to what extent, rather than *how many*, and accordingly it has been adopted here.

* Present address: C.S.I.R.O. Western Australian Regional Laboratory, Nedlands, W.A.

Clearly, the designation of a minimal or representative sample size implies the existence of a reasonably homogeneous area which it may represent. Homogeneity is a concept which has been much discussed in the ecological literature, but without reaching an agreed result. One of the most satisfactory published definitions seems to be that of Dahl and Hadač (1949): "A plant species is said to be homogeneously distributed within a certain area if the probability to catch an individual . . . within a test area of given size is the same in all parts of the area"; without modification, though, it is doubtful whether this definition can be said to cover mosaic patterns. Clearly mosaic types of distribution, though with a lower level of homogeneity than random distribution or the contagious distributions arising from reproductive habits, can still lay claim to homogeneity in so far as samples taken in different parts of the area will give the same results (subject only to random variation) *if they are large enough*. The nub of the question seems to lie in this last phrase, and accordingly I propose as a working but far from formal definition of vegetational homogeneity the following: the distribution of a species in an area of vegetation is homogeneous if there exists some sample size for which the variation between replicate samples is independent of the distance between them. If there is a mosaic pattern, for instance, samples smaller than the component units of the mosaic will resemble adjacent samples more than samples further away which are likely to fall into one of the contrasting components of the mosaic. If samples are about the average size of a mosaic component, the adjacent samples are likely to be more diverse than samples more widely spaced. But if samples are large enough to cover the whole of one recurrent set of components, each will be no more like the next than like one distant from it. The smallest sample area for which, in all species, the variation between replicate samples is independent of the distance between them may be defined as the "minimal area" for the community.

In the past decade, a useful weapon for attacking such mosaic patterns has been introduced from two independent sources into the ecologist's arsenal. This consists in using analysis of variance to study data from a grid of quadrats treated as a hierarchic classification. In 1950 I began collecting such data in Victorian vegetation, and four years later (Goodall 1954*a*) published a first example from a salt marsh. Meanwhile, Greig-Smith (1952*a*) had been examining some model communities in this way, and also (1952*b*) made a first approach to applying the method to observational data (from secondary tropical forest in Trinidad) by using the ratio of the variance between blocks of four plots to that within such blocks as an index of non-random distribution. Since then, he and his students have published several further studies (Phillips 1954; Greig-Smith 1957; Kershaw 1957, 1958, 1959, 1960; Kershaw and Tallis 1958), and Thompson (1955, 1958) has studied statistical aspects of the technique.

In this "grid analysis", data (density or cover) are recorded in a number of quadrats arranged in a grid or transect. The data from adjacent pairs, fours, etc. are combined successively, and the variance is calculated between the two quadrats within each pair, between the two pairs within each four, and so forth. This procedure is analogous to that in a multiple split-plot experiment, no differential treatments being applied, and the interest residing only in comparisons of the estimates of error

variance at the various subplot levels; the plot variance includes, of course, the whole of the variance within subplots, together with any additional variance introduced by comparing subplots within a plot. If the variance estimates are plotted against the size of the groups of quadrats to which they apply, the form of the curve can give valuable information on the scale of patterns existing in the vegetation, a rise in variance indicating that one is passing from one scale of pattern to another. Beyond a size in respect of which the vegetation is homogeneous (Romell's (1926) *Homogenitätsgrenze*) no further increase in variance would be expected. Thus, this technique should provide an unexampled means for determining the minimal area of the community.

The results of "grid analysis" have often (e.g. Greig-Smith 1957) been described as a change in variance with block size. This can be rather misleading. Estimates of such quantities as density, cover, etc. will always show a decrease in variance as the size of sample from a uniform population increases, the variance of the estimate being inversely proportional to the sample size. This is a purely mathematical fact, without ecological interest. But in an analysis of variance, all the variances are expressed in terms of the *same* sample size. The variance between pairs of quadrats within blocks of four, for instance, is not equal to the variance between adjacent quadrats double the unit size; it is equal to the variance between individual quadrats of the unit size, one of which is located within the area occupied by the first pair, the other in that of the second pair. The factor whose effect on variance one is studying is thus not quadrat *size*, but the areas which the quadrats are sampling. And it is not even the size of these areas sampled which is relevant, but their relative position. This will become clear if we consider a hypothetical case where the two areas sampled were overlapping, instead of adjacent as in the usual "grid analysis". If the two unit quadrats compared are placed at random positions within the two overlapping areas, the variance between them will obviously depend on the extent of overlap—or, to put it another way, on the distance between the centres of the areas sampled. Results entirely comparable with those of "grid analysis" could be obtained by independent determinations of variance between unit quadrats spaced randomly within successively larger contiguous areas; "grid analysis" is, however, a more economical means of attaining the same end, since observations on each quadrat contribute to all the variance estimates, and not to one only. In view of these considerations, it seems preferable to refer to the results of "grid analysis" as the changes in variance consequent upon increases in spacing between mean positions of quadrat centres, and in the body of this paper "spacing" will accordingly be used instead of Greig-Smith's "block size".

Greig-Smith and his pupils have claimed that their observations show one, two, or more successive scales of pattern in the vegetation by successive "steps" in the curve connecting variance with quadrat spacing. Doubt has been cast on the statistical significance of many of these "steps" by Thompson, and in respect of my own salt-marsh data I suggested that a continuous curve might fit the observations as well as a "stepped" curve. The present paper presents more data on the course of increase in variance with increase in distance between quadrat centres, in the hope of illuminating further the nature of quantitative pattern in vegetation and possibly aiding in the determination of minimal area.

Strictly speaking, a "stepped" curve of the type postulated would indicate a minimal *intercept* rather than a minimal *area*, since the measure of spacing used is a linear one. In the usual types of vegetation where pattern is isodiametric rather than oriented, this is clearly appropriate. In order that non-random variation in the vegetation should be adequately covered by the sampling unit, it is only important that one dimension of the unit should be long enough to display the pattern; the other dimension merely affects the degree of replication called for. This explains the value of long narrow quadrats which, area for area, give much more information than can be obtained from square or circular quadrats. For historical reasons, however, one may continue to refer to "minimal area", and understand by this the square of the minimal intercept.

Most work on plant distribution has made use of density (number of individuals per unit area) as the measure of quantity. This is not altogether ideal. Apart from the fact that in many species individuals cannot be recognized and counted, individuals do not provide a satisfactory basis for comparison between species varying greatly in stature; and unless one is dealing with an even-aged stand one may find oneself in difficulties within a species too, giving the same weight to individuals differing in size by perhaps several orders of magnitude. Accordingly, instead of density a continuous variable representing the quantity of the species has been preferred—an estimate of ground cover, in fact.

II. Observations and Results

(*a*) *Rhyll Salt Marshes*

Bordering an inlet of Western Port, on the north coast of Phillip Island, are extensive salt marshes. At the water's edge is a pure stand of mangroves (*Avicennia nitida*); on the landward side is a fringe of saline grassland, in which *Distichlis distichophylla* is prominent; and between these communities is a broad belt dominated by *Arthrocnemum halocnemoides*. In this belt, the soil is just covered at high tide. Just above the mangrove zone, the *Arthrocnemum* bushes tend to become less abundant, and there is a corresponding increase in the quantity of *Salicornia australis*; the same thing happens in occasional, slightly lower-lying patches within the *Arthrocnemum* zone. Like most salt marshes, this vegetation is floristically poor. Below the saline grassland zone, only 12 species of Angiosperms were observed.

An apparently uniform area on the southern shore of the inlet within the *Arthrocnemum* zone, 3·2 m square, was selected for the first set of observations in February 1950. The area was divided into 1024 one-decimetre squares, and within each of these the percentage cover by each species was estimated by eye. These percentages were transformed to angular values, and subjected to analysis of variance, to show the changes in variance with increasing distance between quadrats. The results have been tabulated elsewhere (Goodall 1954*a*), and are here reproduced in the form of diagrams (Fig. 1) for the six most abundant species. The quadrats were combined for the purposes of this analysis in groups increasing regularly by a factor of two; but it will be noticed that the successive points are not evenly spaced with reference to the x-axis. This is explained by the spatial distribution of the quadrats. For two adjacent 1-dm quadrats, the distance between their centres is clearly 1 dm.

But when such a pair of quadrats is compared with another such pair with which it forms a square block, the mean distance between the centre of a quadrat in one pair and that of one in the other pair is 1·21 dm. When two adjacent blocks of four are compared, this mean distance becomes 2·13 dm. As combination of quadrats

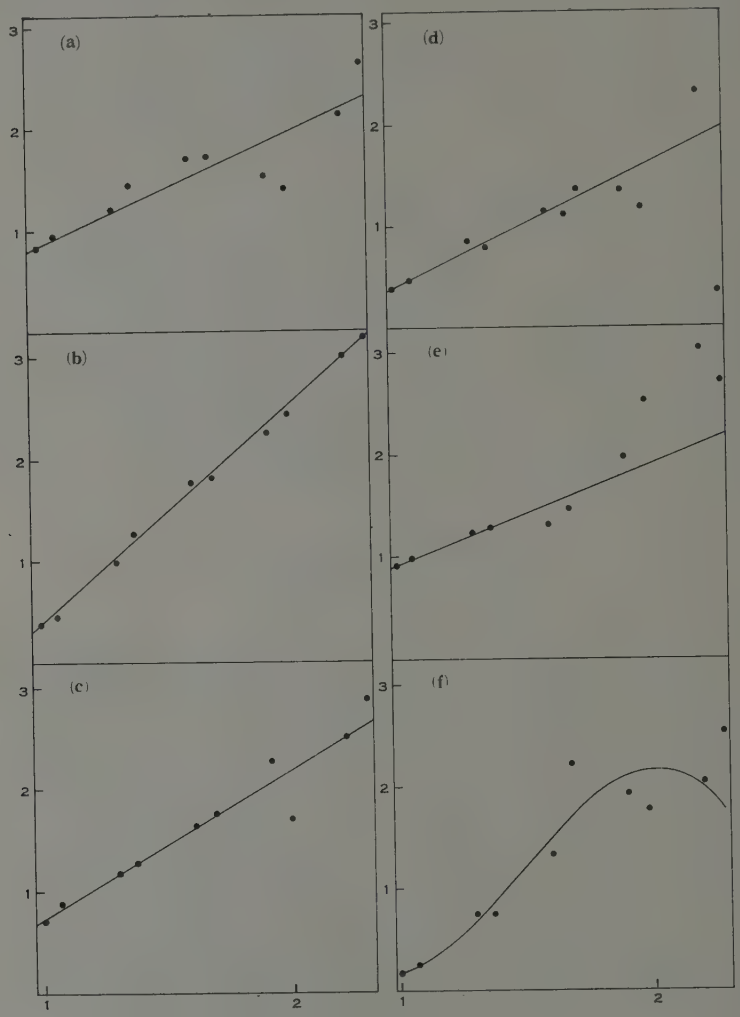

Fig. 1.—The relation of log variance (y-axis) to log spacing in cm (x-axis): Rhyll salt-marsh data, February 1950. (*a*) *Hemichroa pentandra*; (*b*) *Arthrocnemum halocnemoides*; (*c*) *Samolus repens*; (*d*) *Suaeda maritima*; (*e*) *Triglochin striata*; (*f*) *Salicornia australis*.

proceeded the factor by which mean spacing increased was alternately small and large; it will be noted that significant changes in variance generally correspond with the larger proportionate increases in spacing, as might be expected. Since the scale for spacing in the diagrams is logarithmic, the figure used is not the mean

distance between quadrat centres, but the geometric mean of distances between random points in the respective blocks.*

It will be seen from Figure 1 that there was, within these data, no spacing at which the variance between replicates ceased to increase for all species—in other words, there was no evidence of a minimal area in our sense. It was thought that this might have been due to the rather limited area studied, i.e. that a minimum area as defined here existed in this vegetation, but that it was either larger than the whole area (3·2 m square) studied here or sufficiently near to this area not to be ascertainable with the small number of degrees of freedom available for the wider spacings. Accordingly, it was thought desirable to make observations over a much larger area of the same vegetation.

These further observations (performed in May 1950) did not take the form of a grid of quadrats, but quadrats were arranged in a row or transect in order that the same observational labour might cover a wider range of spacing distances. This transect ran roughly parallel with the tidemarks through the *Arthrocnemum* zone, near the site of the previous set of observations. In a belt 1 dm wide, the percentage cover by each species was estimated in each successive decimetre, over a length of 102·4 m. The results were analysed as before, the data being first subjected to angular transformation, and the variance then being analysed between and within groups of quadrats increasing by successive factors of two. The resulting variance estimates are tabulated in Table 1. Here, as in similar tables presented below, the significance levels indicated are the result of comparing each variance against that for the next smaller spacing, grouped with those for any yet smaller spacings not differing significantly from it. Thus, for *Hemichroa pentandra* the first two variances (113·9 and 129·9) did not differ significantly, so they were combined to give a variance estimate of 119·2. The third variance compared with this gave a variance ratio of 1·70, with a significance level of $P < 0{\cdot}001$.

It will be noted in Table 1 that no increase in spacing above 8 dm is accompanied by significant increase in variance for more than half the species. On the other hand, there is no increase in spacing (except that between 256 and 512 dm, where the degrees of freedom are very few) which does not correspond with a significant increase in variance for at least one species. In other words, these observations do not result in an unequivocal demonstration of a "minimum area" for this community, even where large spacings are studied.

In view of the failure of the observations to accord with the stepwise increase of variance with spacing which is to be expected if a minimal area exists, the possibility was considered that the variance is a continuous function of spacing. The type of function that immediately suggested itself was a linear relationship between the

* An expression for the geometric mean distance, x, between points in two adjacent equal rectangles has been kindly derived by Dr. P. White, of Reading University. It is as follows:

$$24 \ln x = 24 \ln b + 32p^2 \ln 2 + 28p \, 2\ln p + 2(p^2+q^2-6) \ln (p^2+1) + (24-16p^2-q^2) \ln (4p^2+1) - \\ -50 + 16(q-p) \tan^{-1} q + 16(4p-q) \tan^{-1}(q/2),$$

where $p = a/b$ and $q = b/a$, b being the length of the common side and a that of the other sides of the two rectangles.

TABLE 1

VARIANCE BETWEEN QUADRATS IN A TRANSECT THROUGH A SALT MARSH, AS A FUNCTION OF THEIR SPACING

Data of May 1950; angular transform of percentage cover

Variance		Degrees of Freedom	*Arthrocnemum halocnemoides*	*Hemichroa pentandra*	*Salicornia australis*	*Samolus repens*	*Suaeda maritima*	*Triglochin striata*
Between (dm)	Within (dm)							
1	2	512	333·7	113·9	215·3	93·0	43·5	8·6
2	4	256	812·7***	129·9	426·2***	174·0***	110·6***	7·0
4	8	128	2072·7***	202·8***	905·9***	269·1***	130·3	7·9
8	16	64	2850·0	378·7**	1799·2***	254·0	174·2*	7·4
16	32	32	2897·9	496·4	1592·8	678·7***	170·1	12·9*
32	64	16	3130·4	690·4	1596·4	1577·8*	109·6	9·1
64	128	8	7548·8*	1462·0**	3137·6	1855·4	214·2	19·3
128	256	4	5191·5	1382·6	520·8	2261·8	734·2**	21·6
256	512	2	597·5	249·0	13887·5***	12857·0**	891·5	78·6**
512	1024	1	1321·5	2795·8	94·0	1281·0	129·4	18·9
Bartlett's test, χ^2			387·47***	186·09***	345·36***	401·07***	157·15***	25·35**

* $P = 0{\cdot}01$–$0{\cdot}05$; ** $P = 0{\cdot}001$–$0{\cdot}01$; *** $P < 0{\cdot}001$.

logarithms of the two quantities, i.e. $\eta_x = \alpha + \beta x$, where $\eta_x = \log \sigma_x^2$, σ_x^2 being the variance between values yielded by a mean distance the logarithm of which is denoted by x; this mean distance is the geometric mean defined above. α and β are natural constants, treated as statistical parameters.

A procedure was accordingly sought for fitting regressions of this type, and the method described in Appendix I was used, as suggested by Dr. F. E. Binet. If η_x is related linearly (or by some more complex regression) to x, then the logarithm of the variance estimate derived from the observational data will not itself be so related, but an adjusted value y_j (the correction depending on the degrees of freedom) will show the same relation. Tables 2 and 3 show the results of fitting regressions to the two sets of data from Rhyll. In these and similar tables presented below, only the "mean square" column of each analysis of variance is presented, and the regression mean square is analysed into that part which can be accounted for by a linear regression, the additional variance accounted for by a quadratic (parabolic) regression, and so forth. Each of these mean squares, together with the residual mean square due to the deviations from the highest regression fitted, may be compared with the error mean square to assess its significance.

In the data of February 1950 (Table 2), it will be seen that in five of the six species, the residual variance after fitting a linear regression was non-significant. In *Salicornia australis*, however, even a quartic regression failed to account satisfactorily for the whole of the variance between spacings. The computed regression lines are shown in Figure 1.

In the data of May 1950 (Table 3), for two of the six species a linear regression adequately accounted for the difference observed; for *Arthrocnemum* and *Triglochin* the quadratic term was significant, for *Suaeda maritima* the quadratic and cubic terms, while for *Salicornia australis* again even a quartic regression left a significant residue. The computed regression lines are shown in Figure 2. It is worth noting that, even where the linear regression was not a fully satisfactory interpretation of the data, it accounted for much the greatest part of the differences between spacings—for over 80% of the variance, in fact.

The significance of the quadratic term in *Arthrocnemum halocnemoides* in Table 3 is reflected in the curvature of the regression line in Figure 2. With spacings above 50 cm, variances show little change; a "plateau" is reached (corresponding with the maximum of the fitted parabolic curve). This would suggest that *Arthrocnemum* is distributed with a minimal area corresponding with a spacing of 50 cm or a little more. If this result is considered further, its interpretation becomes clear. This minimal area is the area occupied by an individual in a closed stand—an individual bush of *Arthrocnemum* commonly measures between 50 and 100 cm across. Much the same applies to *Suaeda*—another species which shows significant deviations from linearity, and the suggestion of a plateau from 36 cm upwards—though here the situation is complicated by a further increase in variance with spacings above about 5 m. In the case of *Salicornia*—a herb of which many individuals could be included in the 1-dm recording unit—the significant quadratic term and the suggested plateau above 71 cm can hardly be explained in the same way as for the two woody species. However, it seems likely that its distribution might be related inversely

TABLE 2

REGRESSION OF LOG VARIANCE ON LOG SPACING, RHYLL SALT MARSHES

Data of February 1950

	Samolus repens	*Arthrocnemum halocnemoides*	*Hemichroa pentandra*	*Salicornia australis*	*Triglochin striata*	*Suaeda maritima*
Regression variance						
Linear	0·83736***	1·75773***	0·46394***	1·44071***	0·34853***	0·53047***
Quadratic				0·00364		
Cubic				0·03525**		
Quartic				0·00120		
Residual variance between spacings	0·00336	0·00450	0·00572	0·01463**	0·00611	0·00506
Error variance	0·00372	0·00372	0·00372	0·00372	0·00372	0·00372

** $P = 0{\cdot}001$–$0{\cdot}01$; *** $P < 0{\cdot}001$.

TABLE 3

REGRESSION OF LOG VARIANCE ON LOG SPACING, RHYLL SALT MARSHES

Data of May 1950

	Samolus repens	*Arthrocnemum halocnemoides*	*Hemichroa pentandra*	*Salicornia australis*	*Triglochin striata*	*Suaeda maritima*
Regression variance						
Linear	0·79652***	1·20315***	0·45355***	0·95795***	0·00198	0·48542***
Quadratic		0·14170***		0·08462***	0·05631***	0·06274***
Cubic				0·00001		0·06352***
Quartic				0·00624		
Residual variance between spacings	0·00604	0·00397	0·00376	0·00901*	0·00372	0·00627
Error variance	0·00372	0·00372	0·00372	0·00372	0·00372	0·00372

* $P = 0{\cdot}01$–$0{\cdot}05$; *** $P < 0{\cdot}001$.

to that of the dominant, and this would cause it to show a degree of patchiness of distribution on the same scale as the individuals of *Arthrocnemum*. On the other hand, the graphs for *Samolus* and *Hemichroa* in Figure 2 show no tendency to a "plateau", and their distribution is presumably independent of the density of shrub foliage or roots. In *Triglochin* the parabolic curve suggests a nearly uniform variance up to 1 m or so, but a continuous increase above this.

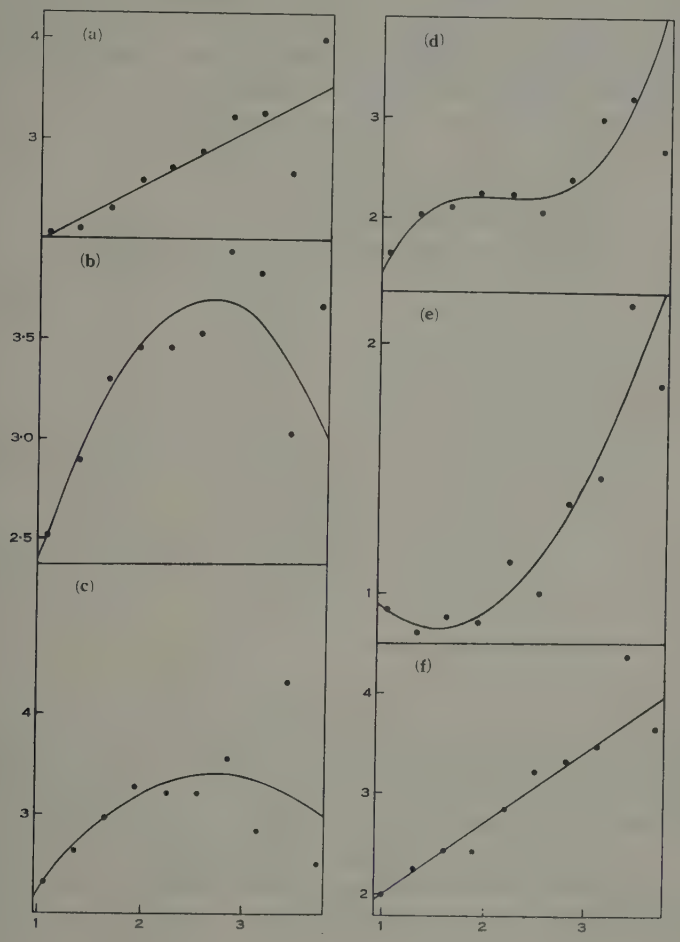

Fig. 2.—The relation of log variance (y-axis) to log spacing in cm (x-axis): Rhyll salt-marsh data, May 1950. (*a*) *Hemichroa pentandra*; (*b*) *Arthrocnemum halocnemoides*; (*c*) *Salicornia australis*; (*d*) *Suaeda maritima*; (*e*) *Triglochin striata*; (*f*) *Samolus repens*.

(*b*) *Victorian Mallee*

The next set of observations comes from the Victorian Mallee near Walpeup, in semi-desert scrub vegetation of which the most conspicuous elements are the low-growing many-stemmed *Eucalyptus* species which give the vegetation its name,

and the large tussocks of porcupine grass, *Triodia irritans*. This was initially studied (in September 1950) by a line transect in the county of Millewa, 1536 m long, running north from near the point where this county joins those of Weeah and Karkarooc. At this stage in the work, it had become clear that to record quadrats spread continuously or regularly over an area (or similarly along a line) was uneconomical of effort for the present purpose, since the precision with which variances were estimated for the smaller spacings was so much greater than for the larger ones. Accordingly, the transect was not studied in its entirety; it was divided into sections 96 m long, and in each section four lengths of 8 m were recorded. These four units were placed in such a way that (*a*) each was separated from any other by at least 4 m (this preventing the same individual being recorded in two units); (*b*) two units

TABLE 4

ANALYSIS OF VARIANCE, PERCENTAGE COVER BY EUCALYPTUS DUMOSA

Data of September 1950; angular transformation

Variance		Degrees of Freedom	Sum of Squares	Mean Square	*F*
Between (m)	Within (m)				
768	1536	1	7·98	7·98	—
384	768	2	54·14	27·07	1·38
192	384	4	78·72	19·68	2·89*
96	192	8	47·21	5·90	1·18
48	96	16	119·04	7·44	1·11
24	48	16	115·31	7·21	1·16
12	24	16	99·89	6·24	—

* $P = 0{\cdot}01 - 0{\cdot}05$.

occurred in each half of the 96-m section; (*c*) in one of these pairs, the two units were placed in different quarters of the 96-m section; (*d*) both the units of the other pair were placed in the same quarter-section. The three degrees of freedom among these four units thus contributed to the variance estimates for different spacings—the difference for pair (*d*) to a spacing (between unit centres) of 12 m, that for pair (*c*) to a spacing of 24 m, and the difference between the means for the two pairs to a spacing of 48 m. Within the scheme of restrictions outlined, the units were placed at random.

Observations took the form of visual estimates of line intercepts, in centimetres of continuous cover for each angiosperm species. Cover was treated as continuous when it contained no more than the normal proportion of gaps within the foliage of an individual of that species. From these intercept estimates, cover percentages were calculated.

An analysis of variance for *Eucalyptus dumosa*, the most important species, is presented in Table 4, and in Table 5 a summary is given of the analyses of variance for the 8 species occurring in at least 10 of the 96 recording units.

TABLE 5

VARIANCE BETWEEN TRANSECT LENGTHS AT DIFFERENT SPACINGS: VICTORIAN MALLEE

Data of September 1950

	Geometric Mean Spacing (m)							Bartlett's Test χ^2
	11·5	21·8	42·6	85·1	170·0	340·0	680·0	
Bassia uniflora	0·42	0·84	0·79	0·32	4·83***	2·89	17·01	31·32***
Dodonaea bursariifolia	1·65	2·15	1·61	1·55	7·56**	2·13	6·63	7·46
Eucalyptus calycogona	5·51	2·84	1·20	2·84	9·97*	7·37	0·43	15·00*
Eucalyptus dumosa	6·24	7·21	7·44	5·90	19·68*	27·07	7·98	5·44
Eucalyptus oleosa	1·87	8·88**	5·89	15·41	22·34	20·25	5·88	18·17**
Leptospermum coriaceum	1·12	2·59	2·32	4·77*	0·92	31·08**	43·56	31·99***
Melaleuca uncinata	1·76	5·70*	7·38	8·58	28·18**	16·97	27·04	19·52**
Toxanthus muelleri	0·33	0·25	1·06**	0·64	0·20	1·79	0·39	13·67*
Triodia irritans	3·36	1·70	4·52	5·34	6·29	2·51	15·31*	7·28

* $P = 0{\cdot}01$–$0{\cdot}05$; ** $P = 0{\cdot}001$–$0{\cdot}01$; *** $P < 0{\cdot}001$.

TABLE 6

REGRESSION OF LOG VARIANCE ON LOG SPACING: VICTORIAN MALLEE

Data of September 1950

	Bassia uniflora	*Eucalyptus calycogona*	*Eucalyptus oleosa*	*Leptospermum coriaceum*	*Melaleuca uncinata*	*Toxanthus muelleri*
Regression variance						
Linear	0·31222**	0·00163	6·12802***	0·35601**	0·79812***	0·17671*
Quadratic		0·29717*				
Residual variance between spacings	0·09360	0·07402	0·05314	0·08208	0·02474	0·07653
Error variance	0·04648	0·04648	0·04648	0·04648	0·04648	0·04648

* $P = 0{\cdot}01$–$0{\cdot}05$; ** $P = 0{\cdot}001$–$0{\cdot}01$; *** $P < 0{\cdot}001$.

For most of the species, the variances differed with spacing (see the results of Bartlett's test in Table 5), but the differences, apart from a clear tendency to increase with spacing, follow no clear pattern. No point in the spacing scale corresponds with a variance increase for all the species listed; and, on the other hand, every increase in spacing is accompanied by a significant increase in variance for at least one species. In other words, no clear "minimum area" for the vegetation as a whole is indicated.

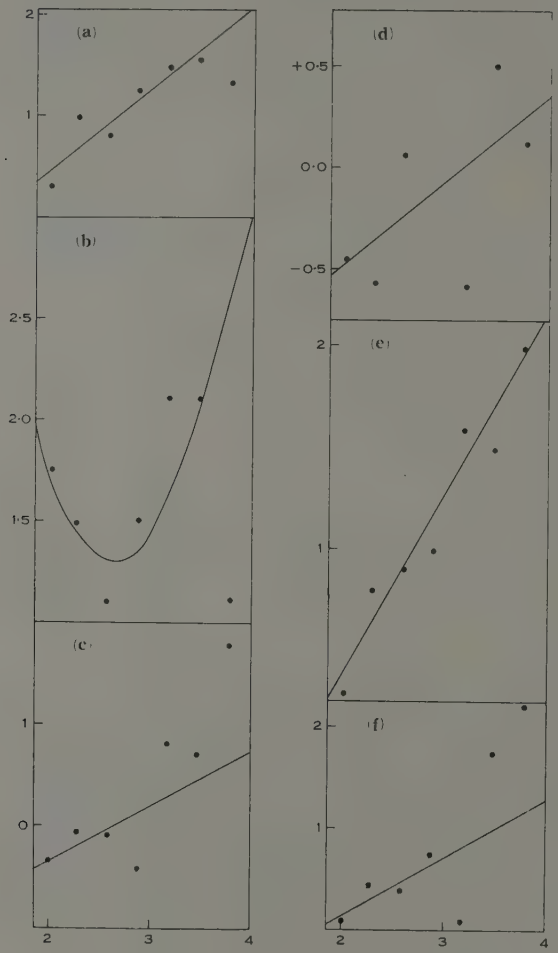

Fig. 3.—The relation of log variance (y-axis) to log spacing in cm (x-axis): Mallee data, September 1950. (*a*) *Eucalyptus oleosa*; (*b*) *E. calycogona*; (*c*) *Bassia uniflora*; (*d*) *Toxanthus muelleri*; (*e*) *Melaleuca uncinata*; (*f*) *Leptospermum coriaceum*.

Once again, the alternative hypothesis of a continuous increase in variance with spacing has to be tested. The results of the test are presented in Table 6 and Figure 3. It will be seen that a linear regression accounted satisfactorily for the differences in variance with spacing in all species except *Eucalyptus calycogona*, for which a quadratic regression was appropriate.

In the course of the field work on this transect, it had become clear that line intercepts did not provide an ideal method of measuring cover in this vegetation. Moreover, the number of units studied was hardly large enough, considering the range of variation encountered in the vegetation (certain species were completely absent from one half of the transect line, frequent in the other half). With a view to obtaining more adequate information about the structure of this vegetation, another set of observations was made in December 1950. These observations have already been used in other connexions (Goodall 1953, 1954*b*), but were primarily intended and designed for the present purpose. The units of observation were quadrats 5 m square. An area 640 m square was divided into 64 sections 80 m square, in each of which four

TABLE 7

ANALYSIS OF VARIANCE: PERCENTAGE COVER BY EUCALYPTUS DUMOSA

Data of December 1950; angular transformation

	Degrees of Freedom	Sum of Squares	Mean Square	*F*
Between adjacent 320 × 640-m rectangles	1	290	290	—
Between adjacent 320-m squares	2	7283	3641	5·25*
Between adjacent 160 × 320-m rectangles	4	5846	1461	2·58
Between adjacent 160-m squares	8	1377	172	—
Between adjacent 80 × 160-m rectangles	16	12201	763	3·13***
Between adjacent 80-m squares	32	6458	202	—
Between 40-m within 80-m squares	64	16915	264	2·00***
Between 20-m within 40-m squares	64	8464	132	—
Between 10-m within 20-m squares	64	8492	133	—

* $P = 0{\cdot}01-0{\cdot}05$; *** $P < 0{\cdot}001$.

quadrats were located. Each of the 64 sections was divided into 64 plots 10 m square, and four of these plots were selected for sampling as follows:

The section was divided into quarters, and two of these quarters were selected at random. Each of these quarters (40 m square) was further divided into four; in one, one of the 20-m squares was selected at random, and two 10-m plots within it taken at random for sampling; in the other 40-m square, two of the 20-m squares were selected at random, and one plot for sampling located in each of them. The quadrats were located in each case in the south-east corner of the 10-m plot, thus ensuring a gap of at least 5 m between adjacent quadrats. This arrangement provided, in two dimensions, the same facilities as had been achieved by the restricted randomization described above for the line transect—the variances for the three smallest spacings studied could be estimated with equal precision.

A sketch map of each quadrat was drawn, showing the areas covered by foliage of the larger species; from these sketch maps, estimates of cover were subsequently obtained. For smaller plants, direct eye estimates of cover were made. Before analysis of variance, the data for percentage cover were subjected to angular transformation.

TABLE 8

VARIANCE BETWEEN PERCENTAGE COVER AS A FUNCTION OF SPACING IN THE VICTORIAN MALLEE

Data of December 1950; angular transformation

	Geometric Mean Spacing (m)									Bartlett's Test χ^2
	11	23	45	81	95	161	191	322	381	
Bassia uniflora	2·1	3·0	5·6***	7·9	23·2***	9·0	60·3*	14·2	15·8	84·04***
Dodonaea bursariifolia	18·4	29·5*	56·6**	50·7	50·9	79·3	450·7***	11·4	428·0	57·38***
Eucalyptus calycogona	68·6	99·8	71·9	88·9	74·0	172·4**	329·1	1499·3*	149·9	33·67***
Eucalyptus dumosa	132·7	132·2	264·3***	201·8	762·5***	172·1	1461·5	3641·3*	290·1	65·59***
Eucalyptus oleosa	158·8	251·7*	319·3	360·4	895·0**	262·0	3129·9**	1876·2	1669·2	55·01***
Melaleuca uncinata	38·0	127·9***	134·8	131·9	363·7***	574·1	1475·4*	1898·1	657·9	97·42***
Stipa variabilis	3·2	1·4	1·5	3·6**	3·9	8·3*	2·3	0·2	107·0***	64·20***
Triodia irritans	50·4	41·9	130·2***	175·5	517·5***	162·0	1061·7	550·5	1778·2	102·06***
Vittadinia triloba	2·6	6·6***	7·9	9·7	4·2	28·0***	30·9	39·9	58·4	54·34***
Zygophyllum apiculatum	0·5	0·8	1·7***	2·0	4·0**	1·9	10·2*	7·0	0·2	65·67***

* $P = 0{\cdot}01$–$0{\cdot}05$; ** $P = 0{\cdot}001$–$0{\cdot}01$; *** $P < 0{\cdot}001$.

The analysis of variance is illustrated in Table 7, using the data for *Eucalyptus dumosa*, the commonest species. In Table 8 a summary is given of the analyses of variance for the 10 commonest species. Again, it will be seen that there is a general tendency for the variance to increase with spacing, but that at no particular spacing does the increase cease for all species. In other words, there is no evidence for a minimal area by our criteria, within the range of spacing tested; if a minimal area exists, it is less than 10 m square and could contain very few individuals of the dominant species.

TABLE 9

REGRESSION OF LOG VARIANCE ON LOG SPACING: VICTORIAN MALLEE

Data of December 1950

	Regression Variance				Residual Variance between Spacings	Error Variance
	Linear	Quadratic	Cubic	Quartic		
Bassia uniflora	0·80640***				0·02216	
Dodonaea bursariifolia	0·42143***				0·02100	
Eucalyptus calycogona	0·05580*				0·02494	
Eucalyptus dumosa	0·31399***	0·02685	0·00020	0·03423	0·05198**	
Eucalyptus oleosa	0·36656***				0·02249	
Melaleuca uncinata	0·87333***	0·00171	0·14497**		0·02023	0·01376
Stipa variabilis	0·01360	0·23485***	0·10924**		0·02858	
Triodia irritans	0·85737***	0·06612*	0·04744	0·05290*	0·03239	
Vittadinia triloba	0·47799***	0·00384	0·12059**		0·02013	
Zygophyllum apiculatum	0·74692***				0·01557	

* $P = 0·01-0·05$; ** $P = 0·001-0·01$; *** $P < 0·001$.

Again, regressions were computed, and the results are seen in Table 9 and Figure 4. In 5 of the 10 species a linear regression accounts satisfactorily for the spacing differences. In other cases more complex regressions are shown (for one species beyond quartic), and there is no consistent pattern.

This area of mallee vegetation, though falling within a single community type recognized by ecologists working in the area (Beadle 1948; Zimmer 1946), appeared to be heterogeneous when studied in detail, and four distinct communities had been recognized within it following an objective analysis (Goodall 1953). It might be argued that the continuous increase in variance demonstrated was due to the data covering these distinct (though intergrading) communities rather than being located within a single community. It was accordingly thought worth while to study the changes in variance within three of these communities (the fourth was not well enough represented to provide adequate data). In view of the irregular shape of the areas occupied by each community, the degrees of freedom available for different spacings did not constitute a regular series, and there were only six variance estimates available for each species. The results are shown in Tables 10 and 11 and Figure 5.

In certain cases, the variance estimates did not differ among themselves. In the *Vittadinia* community, only the variance for the smallest spacing differed significantly from the others in the data for *V. triloba* itself, and for *Stipa variabilis* the

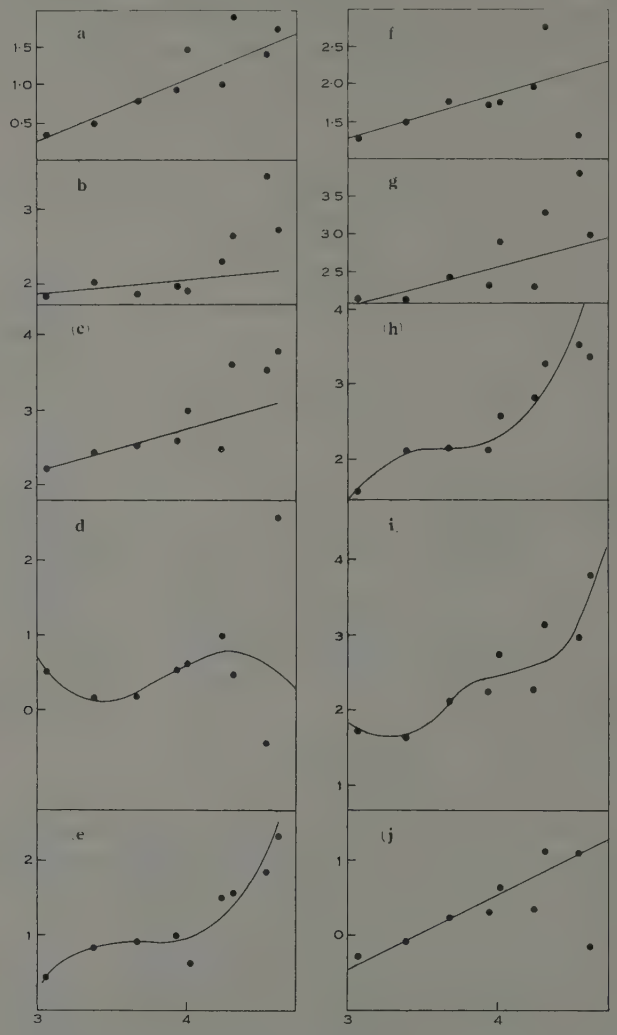

Fig. 4.—The relation of log variance (y-axis) to log spacing in cm (x-axis): Mallee data, December 1950. (*a*) *Bassia uniflora*; (*b*) *Eucalyptus calycogona*; (*c*) *E. oleosa*; (*d*) *Stipa variabilis*; (*e*) *Vittadinia triloba*; (*f*) *Dodonaea bursariifolia*; (*g*) *E. dumosa*; (*h*) *Melaleuca uncinata*; (*i*) *Triodia irritans*; (*j*) *Zygophyllum apiculatum*.

variance for the smallest spacing was notably high; these deviations are reflected in significance of the quadratic and cubic terms in Table 11. In the other communities, three of the species tested showed a significant linear regression which satisfactorily

TABLE 10

VARIANCE OF PERCENTAGE COVER AS A FUNCTION OF SPACING: DISTINCT COMMUNITIES IN THE VICTORIAN MALLEE

Data of December 1950

(*a*) Dodonaea *Grouping*

Geometric Mean Spacing (m)	Degrees of Freedom	*Dodonaea bursariifolia*	*Eucalyptus dumosa*	*Melaleuca uncinata*	*Triodia irritans*
11	16	20·2	252·5	48·0	41·1
23	15	61·1*	123·5	104·1	39·6
45	13	35·5	178·0	58·5	129·8
81	11	89·1	56·1	180·9*	123·3
161	8	163·5*	110·4	501·2	71·6
322	3	239·4	269·7	24·9	23·8
Bartlett's test, χ^2		18·84**	7·86	21·80***	10·27

(*b*) Bassia *Grouping*

Geometric Mean Spacing (m)	Degrees of Freedom	*Bassia uniflora*	*Eucalyptus oleosa*	*Zygophyllum apiculatum*
11	22	48·7	261·8	1·6
23	20	56·6	221·2	1·2
45	21	54·0	282·0	3·6**
81	21	97·8*	443·7*	3·8
161	11	100·4	1001·9	3·0
322	1	180·9	420·2	20·1*
Bartlett's test, χ^2		5·95	12·28*	13·85**

(*c*) Vittadinia *Grouping*

Geometric Mean Spacing (m)	Degrees of Freedon	*Stipa variabilis*	*Vittadinia triloba*
11	20	5·0	2·0
23	14	0·7	12·6***
45	18	1·4	6·7
81	14	2·6	5·2
161	8	5·2	12·1
322	4	12·1**	30·4*
Bartlett's test, χ^2		24·64***	22·72***

* $P = 0·01-0·05$; ** $P = 0·001-0·01$; *** $P < 0·001$.

accounted for the differences among the variance estimates, while in *Melaleuca uncinata* higher terms had to be fitted. The general picture is thus not dissimilar from that obtained when the whole area was considered together.

(c) *Uganda Rain-forest*

Next, we have a valuable set of data, very kindly made available by H. C. Dawkins of the Forestry Department in Uganda. These data were collected in the Mabira forest, Kyabana block, near the road from Jinja to Bugerere. An area of forest measuring 4 miles (6·44 km) by 2 miles (3·22 km) was sampled by quadrats spaced along parallel transects running across the narrower dimension. The transects

TABLE 11

REGRESSION OF VARIANCE ON SPACING IN DISTINCT COMMUNITIES IN THE VICTORIAN MALLEE

Data of December 1950

	Regression Variance				Residual Variance between Spacings	Error Variance
	Linear	Quadratic	Cubic	Quartic		
Dodonaea grouping						
Dodonaea bursariifolia	0·53484***				0·03572	0·03755
Melaleuca uncinata	0·28241**	0·01591	0·09949	0·29809**	0·01496	
Bassia grouping						
Eucalyptus oleosa	0·18666**				0·01703	0·02500
Zygophyllum apiculatum	0·18304**				0·03162	
Vittadinia grouping						
Stipa variabilis	0·04378	0·50707***			0·07288	0·03158
Vittadinia triloba	0·34276***	0·00367	0·27366**		0·04958	

** $P = 0·001-0·01$; *** $P < 0·001$.

were sited randomly, one within each quarter-mile segment of the base line. The quadrats, too, were located by restricted randomization, two within each quarter-mile length of transect. The minimum spacing between quadrats was thus considerably greater than in the other sets of data recorded above—even taking into account the different scale of the vegetation—and substantially above the "minimal area" of 150 sq. m. (by species–area curve) reported for similar vegetation in the Ivory Coast (Emberger, Mangenot, and Miège 1950*a*, 1950*b*).

Each quadrat measured 44×22 yd (40·2×20·1 m). Within it there were recorded, for each of 29 species (the most important tree species present), the numbers in each girth class from 2 ft upwards, i.e. the number of individuals whose girth at breast height fell within the ranges 18–30 in. (45·7–76·2 cm), 30–42 in. (76·2–106·7 cm), etc. Further, saplings less than 6 in. in girth but exceeding 6 ft (1·83 m) in height, and young trees in the 1 ft girth class (girth between 6 in. and 18 in. (15·2 and 45·7 cm)), were counted up to a limit of six, and above that recorded simply as "numerous"; the presence of seedlings (less than 1 ft (30·5 cm) high) and young saplings (between 1 ft and 6 ft high) was also recorded.

The most appropriate way to analyse these data seemed to be in terms of basal area. It was accordingly necessary to convert the records by girth classes into basal areas. This was done as follows, for each species separately: Saplings and seedlings were ignored. The mean number of trees in the 1 ft girth class, where this was recorded simply as "numerous", was estimated by plotting a frequency curve

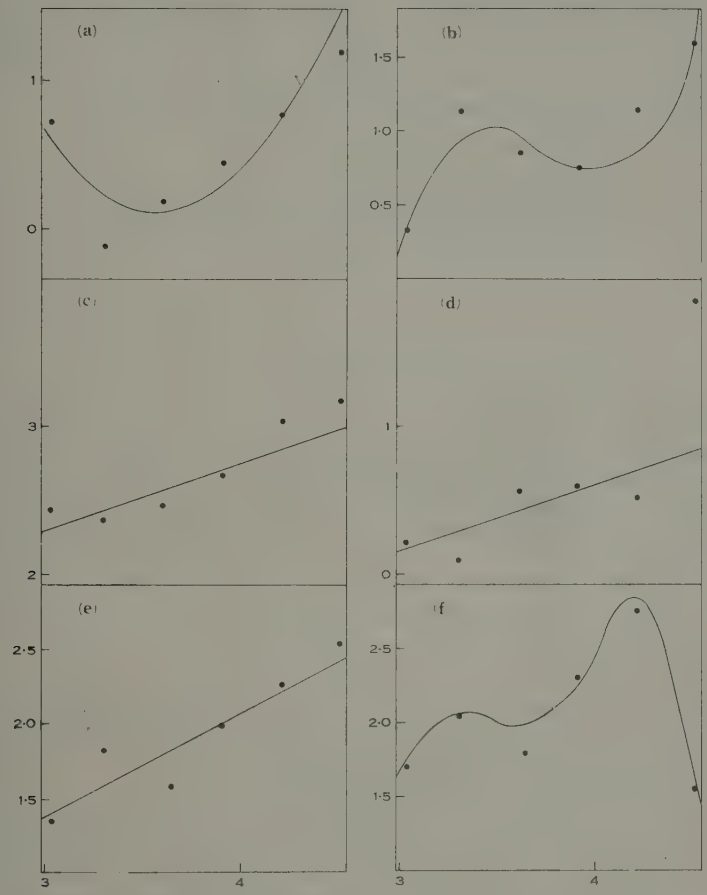

Fig. 5.—The relation of log variance (y-axis) to log spacing in cm (x-axis): distinct communities in the Victorian Mallee, December 1950 (Table 10). *Vittadinia* grouping: (*a*) *Stipa variabilis*; (*b*) *Vittadinia triloba*. *Bassia* grouping: (*c*) *Eucalyptus oleosa*; (*d*) *Zygophyllum apiculatum*. *Dodonaea* grouping: (*e*) *Dodonaea bursariifolia*; (*f*) *Melaleuca uncinata*.

for quadrats with 1, 2, etc. trees in this category and extrapolating. Another frequency curve was then prepared in which the number of individuals in a girth class was shown as a function of the girth, the estimated mean for the 1 ft girth class being used wherever the record was "numerous". This curve enabled the distribution of girths within each girth class to be estimated, and hence the average basal area

for that class, treating the girth as the circumference of a circle. From these averages, and the number of trees in each class, the basal area for each quadrat could then be readily computed.

Of the 256 quadrats, 5 fell in grassland enclaves within the forest and 11 fell in a coffee plantation. These were treated as missing plots in the subsequent analysis.

For each analysis of variance, the two plots within a quarter-mile length of transect were first combined. This pair was then combined with an adjacent pair along the transect, these four with the corresponding four on the next transect, and so on, alternately combining along and across the transect direction. The basal area data were subjected to square root transformation before analysis.

TABLE 12

CELTIS BROWNII IN THE MABIRA FOREST: ANALYSIS OF VARIANCE

Basal area (sq.m./ha) after square root transformation

	n	Sum of Squares	Mean Square	F
Between adjacent 2-mile squares	1	19·486	19·486	10·79**
Between adjacent strips 1 mile wide	2	8·531	4·266	3·06
Between adjacent 1-mile squares	4	3·226	0·807	—
Between 1-mile lengths of adjacent pairs of transects	8	13·498	1·687	3·62***
Between adjacent ½-mile squares	16	10·595	0·662	1·54
Between ½-mile lengths of adjacent transects	31	13·600	0·439	1·03
Between adjacent ¼-mile squares	59	25·177	0·427	1·48*
Within ¼-mile squares	118	33·974	0·288	

* $P = 0{\cdot}01-0{\cdot}05$; ** $P = 0{\cdot}001-0{\cdot}01$; *** $P < 0{\cdot}001$.

The results for the 13 most frequent species were analysed in this way. The complete analysis of variance for *Celtis brownii* is presented in Table 12 and the results for the other species are summarized in Table 13.

Bartlett's test (see the last column of Table 13) showed that, for all species but two, the variances differed significantly between spacings. If Table 13 is inspected, it will be noted that nearly half of the significant differences recorded occur in one column—in 9 of the 13 species the variance between the half-mile squares is greater than the variance within them—and that the preceding column is almost empty of asterisks. This is the most definite suggestion of a "minimal area" that we have yet seen. By the criterion adopted, it would appear that an area a quarter of a mile (360 m) square would have some claim to be accepted as the "minimal area" for this forest type. This is an area, to be sure, far larger than any suggested elsewhere in the literature; but tropical forest has rarely been considered in this way.

The minimal area of 150 sq. m. suggested for the Ivory Coast forests on the basis of species–area curves (Emberger, Mangenot, and Miège 1950*a*, 1950*b*) has already been mentioned; and it must be admitted that, if it existed in the Mabira forest, it could not be recognized from these data. This figure for minimal area has,

TABLE 13

VARIANCE BETWEEN QUADRATS AT DIFERENT SPACINGS: MABIRA FOREST

	Geometric Mean Spacing (m)								Bartlett's Test χ^2
	153	360	479	811	960	1619	1917	3241	
Aphania senegalensis	0·159	0·247*	0·284	0·461*	0·792	0·528	0·461	1·238	24·56***
Bosqueia phoberos	0·161	0·155	0·240	0·521***	0·429	0·098	0·039	0·001	24·84***
Celtis brownii	0·288	0·426*	0·438	0·662	1·686***	0·806	4·266	19·490**	64·68***
Celtis kraussiana	0·460	0·598	0·532	0·609	1·141*	1·582	1·949	10·023*	21·79**
Celtis soyauxii	0·621	1·000*	0·536	2·105**	1·876	1·035	1·950	6·990	25·26***
Celtis zenkeri	0·435	0·596	0·511	1·875***	0·942	0·522	1·829	2·220	22·84**
Chrysophyllum albidum	0·246	0·324	0·311	1·103***	2·085	0·436	1·406	31·452***	101·24***
Lasiodiscus mildbraedii	0·158	0·595***	0·386	0·716	0·979	1·020	1·192	1·990	53·35***
Maba abyssinica	0·568	0·806	0·783	1·498***	1·875	3·980	1·000	1·004	21·54**
Markhamia platycalyx	0·688	0·809	0·714	1·267*	1·087	0·884	0·917	0·079	5·25
Melanodiscus sp. nov.	0·183	0·133	0·120	0·335**	0·669	0·226	0·202	1·175	22·28**
Tapura fischeri	0·231	0·285	0·300	0·433*	0·483	0·703	0·176	0·341	7·83
Trichilia prieuriana	0·079	0·079	0·135*	0·211	0·234	0·038	0·183	1·155**	23·54**

* $P = 0·01-0·05$; ** $P = 0·001-0·01$; *** $P < 0·001$.

however, a low *prima facie* credibility from the present point of view, since it is probably smaller than the average area occupied by one individual of the uppermost stratum.

Despite the suggestion of a minimal area in the Mabira data, when they are analysed in the alternative way already described one finds that the hypothesis of continuous increase in variance with spacing also fits them surprisingly satisfactorily (Table 14, Fig. 6). In 8 of the 11 species a linear regression accounted satisfactorily for the differences among spacings, while in the other three species quadratic or cubic regressions were indicated.

TABLE 14

REGRESSION OF LOG VARIANCE ON LOG SPACING: MABIRA FOREST

	Regression Variance			Residual Variance between Spacings	Error Variance
	Linear	Quadratic	Cubic		
Aphania senegalensis	0·29347***			0·00438	
Bosqueia phoberos	0·06109*	0·00518	0·17311***	0·00290	
Celtis brownii	0·28369***			0·01483	
Celtis kraussiana	0·10508**			0·01319	
Celtis soyauxii	0·16409***			0·02033	
Celtis zenkeri	0·19706***			0·02075	0·01301
Chrysophyllum albidum	0·33236***	0·07418*		0·02429	
Lasiodiscus mildbraedii	0·68026***			0·01523	
Maba abyssinica	0·22173***			0·00610	
Melanodiscus sp. nov.	0·02620	0·12247**		0·01833	
Trichilia prieuriana	0·11829**			0·02430	

* $P = 0{\cdot}01-0{\cdot}05$; ** $P = 0{\cdot}001-0{\cdot}01$; *** $P < 0{\cdot}001$.

(*d*) *Welsh Grassland and Moorland*

Some recent publications using "grid analysis" (Kershaw 1958, 1959; Kershaw and Tallis 1958) have included cover observations, and these data have been re-examined in the same way as the original data described above. The results are shown in Table 15 and Figures 7–9. It should be noted that the data were published in the form of diagrams, and that the ordinates could be measured with only limited accuracy—particularly in the case of the smaller variances corresponding with more degrees of freedom. The variance between transects (Kershaw 1958, 1959) has been omitted because, as the author indicates, this could not be expected simply to continue the series formed by the successive variances within a transect.

As may be seen from Table 15, Kershaw's data resemble those already presented in that by far the major part of the differences among the variances can usually be accounted for by a linear regression. On the other hand, in only a relatively small number of cases (4 out of 22) does the linear regression satisfactorily account for the whole variation. In a number of the other cases, the fitted curve could be taken as

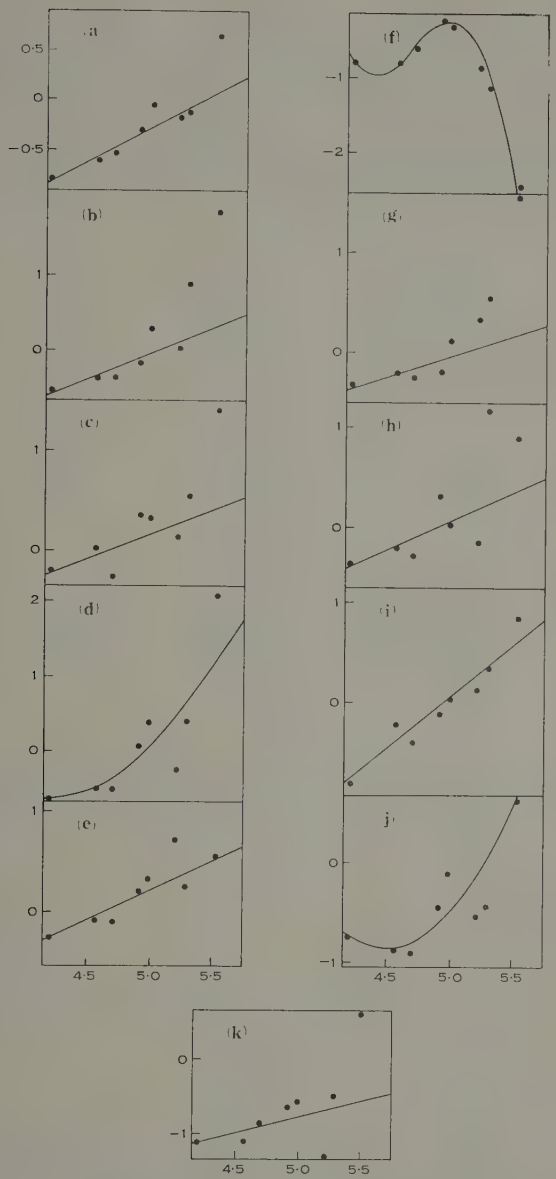

Fig. 6.—The relation of log variance (y-axis) to log spacing in cm (x-axis): Mabira Forest. (*a*) *Aphania senegalensis*; (*b*) *Celtis brownii*; (*c*) *C. soyauxii*; (*d*) *Chrysophyllum albidum*; (*e*) *Maba abyssinica*; (*f*) *Bosqueia phoberos*; (*g*) *Celtis kraussiana*; (*h*) *C. zenkeri*; (*i*) *Lasiodiscus mildbraedii*; (*j*) *Melanodiscus* sp. nov.; (*k*) *Trichilia prieuriana*.

TABLE 15

REGRESSION OF LOG VARIANCE ON LOG SPACING: PUBLISHED DATA

Source	Species†	Regression Variance				Residual Variance between Spacings	Error Variance
		Linear	Quadratic	Cubic	Quartic		
Kershaw (1957)	*Nardus stricta* (Fig. 21)	0·63346***	0·05208***			0·00510	
Kershaw (1958)	*Agrostis tenuis* (Fig. 6)	1·65036***	0·06424***	0·00772	0·00024	0·01523**	
	(Fig. 7)	0·98568***	0·02832***	0·00027	0·05328***	0·00068	0·00262
	(Fig. 8)	0·29331***	0·01053*	0·00037	0·05018***	0·03383***	
	(Fig. 12)	1·64191***	0·12230***	0·03831***		0·00181	
	(Fig. 14)	0·74841***	0·04099***	0·14506***	0·00176	0·05606***	
Kershaw and Tallis (1958)	*Juncus squarrosus* (Fig. 6)	0·12713***				0·00513	
	Festuca ovina (Fig. 7)	0·08671***				0·00241	0·00298
	Galium hercynicum (Fig. 8)	0·26750***	0·02176**	0·02679**		0·00610	
	Agrostis tenuis (Fig. 9)	0·17941***	0·00769	0·00116	0·01006	0·00931*	
	Polytrichum commune (Fig. 10)	0·28898***	0·01020*	0·04423***	0·00010	0·04202***	
Kershaw (1959)	*Dactylis glomerata* (Fig. 1)	0·37541***	0·00063	0·00195	0·00931	0·01118*	
	(Fig. 2)	0·25896***	0·01772**			0·00376	
	(Fig. 3)	0·46596***				0·00565	
	Lolium perenne (Fig. 4)	0·51305***	0·01951**			0·00597	
	(Fig. 5)	0·36855***	0·00959	0·02242**		0·00150	0·00262
	(Fig. 6)	0·63151***	0·00427	0·00045	0·01769*	0·01758**	
	Trifolium repens (Fig. 13)	0·39219***	0·02091**	0·08104***	0·01660*	0·01550**	
	(Fig. 14)	0·06044***	0·02735**			0·00489	
	(Fig. 15)	0·14229***				0·00571	
	(Fig. 16)	0·52828***	0·00424	0·01316*	0·00103	0·01578**	
	(Fig. 17)	0·91144***	0·00001	0·03858**	0·00445	0·02940***	

* $P = 0·01-0·05$; ** $P = 0·001-0·01$; *** $P < 0·001$.

† Figure numbers in this column refer to figures in the papers cited in the previous column.

an approximation to the general "stepwise" hypothesis favoured by Kershaw—though it would often not support the particular "steps" he recognizes. For instance, the curve for *Galium hercynicum* (Fig. 9(*e*)) does not suggest that the peak at 160 cm, which Kershaw and Tallis regard as meaningful, is anything more than fortuitous.

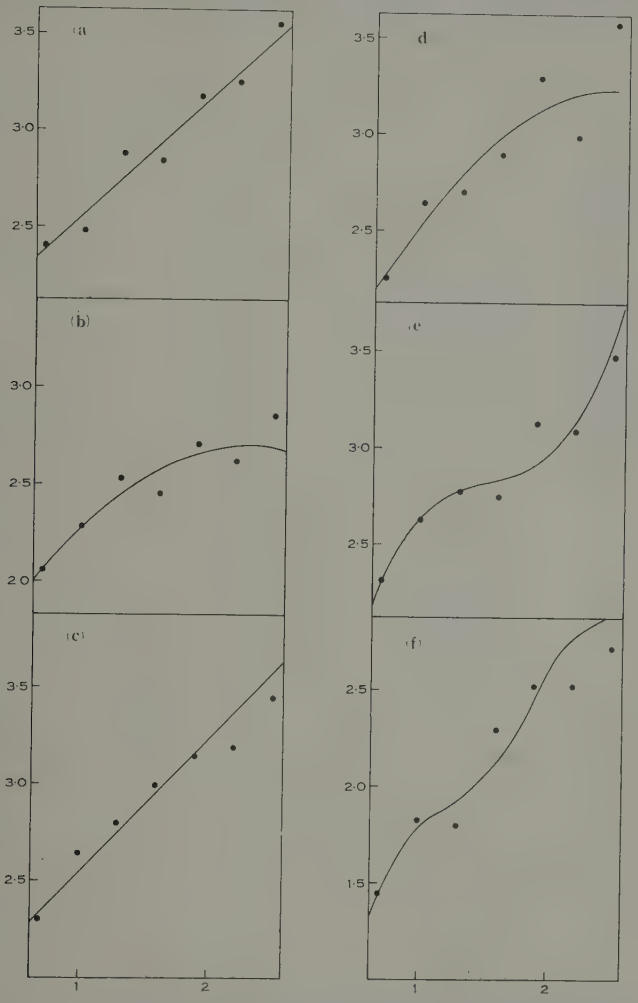

Fig. 7.—The relation of log variance (*y*-axis) to log spacing in cm (*x*-axis): data of Kershaw (1959) for *Dactylis glomerata* ((*a*), (*b*), and (*c*)—Kershaw's Figs. 1–3) and *Lolium perenne* ((*d*), (*e*), and (*f*)—Kershaw's Figs. 4–6).

Similarly, Kershaw takes his data for *Dactylis glomerata* collected from the area of F. Dan Buarth sown in 1945 (Fig. 7(*b*) here) as indicating "peaks" at 20 and 80 cm, whereas the fitted curve only suggests a plateau about 80 cm.

If deviations from linearity in the curve represent a mosaic pattern in the vegetation as a whole, it is to be expected that these deviations will agree approximately as between the different species. This is by no means generally true. In the

data of Kershaw and Tallis for five species (Fig. 9 (*b*)–(*f*)) there is reasonably good agreement between *Polytrichum* and *Galium*, both species showing a "plateau" between spacings of 20 and 80 cm; but the other three species differ. In Kershaw's data from Ffridd Ddu (Figs. 7, 8) four comparisons of results for different species in the same area are possible. Of these four comparisons, one (*Agrostis* and *Lolium*

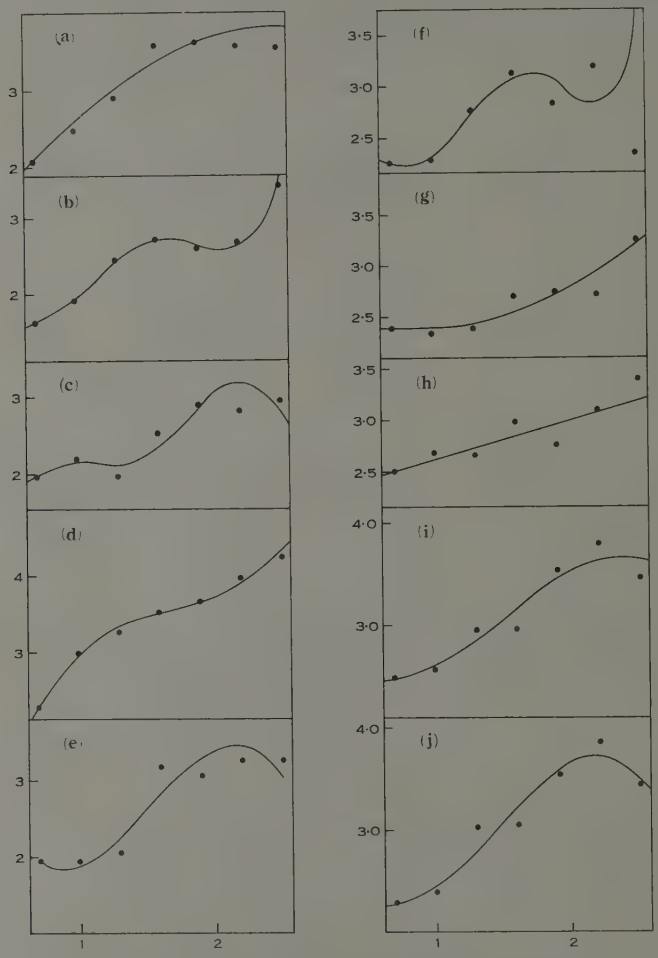

Fig. 8.—The relation of log variance (*y*-axis) to log spacing in cm (*x*-axis): data of Kershaw (1958, 1959) for *Agrostis tenuis* and *Trifolium repens*. (*a*)–(*e*), *Agrostis tenuis* (Kershaw 1958, Figs. 6–8, 12, 14); (*f*)–(*j*), *Trifolium repens* (Kershaw 1959, Figs. 13–17).

on F. F. Lavan 1952(A): Figs. 7(*d*) and 8(*a*)) shows reasonably good agreement, with a parabolic curve approaching a plateau above a spacing of 80 cm. But the other three comparisons (Figs. 7(*b*) and 7(*f*); Figs. 7(*e*) and 8(*h*); Figs. 8(*b*) and 8(*i*)) have quite dissimilar curves.

III. Discussion

The conclusion from these analyses would seem to be that in many instances the variance between records of cover in replicate quadrats increases progressively and continuously as the distance between them increases. Once one gets away from the concept of vegetational homogeneity, this conclusion occasions no surprise—in

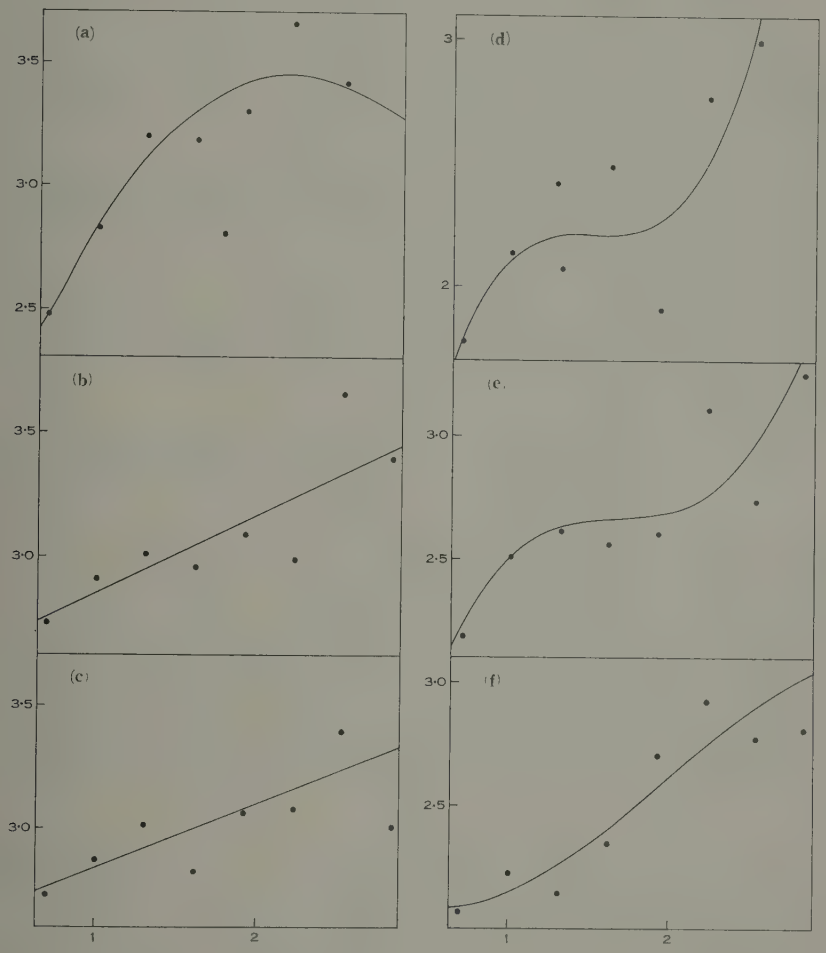

Fig. 9.—The relation of log variance (y-axis) to log spacing in cm (x-axis): data of Kershaw (1957) and Kershaw and Tallis (1958) for (*a*) *Nardus stricta*; (*b*) *Juncus squarrosus*; (*c*) *Festuca ovina*; (*d*) *Polytrichum commune*; (*e*) *Galium hercynicum*; (*f*) *Agrostis tenuis*.

fact, strikes one as fully reasonable. Consider vegetational characters as functions of environmental variables. In general, the latter vary continuously from place to place, so that two points in close proximity are bound to have very similar values, while the possible differences increase as the points of measurement separate. This

is commonplace in respect of rainfall distribution, for instance. The amounts of rain received in a single storm at two points within a metre of one another will agree very closely; at 100 m they may vary by 50%, while at 1 km one of the points may have received 20 mm and the other none at all. The same will be true of other environmental factors. Thus the quantities of plants dependent on the environmental factors could be expected to show comparable continuous increase in variance with distance. On the other hand, a homogeneous environment should be reflected in a plant distribution homogeneous when the scale is large compared with the reproductive pattern. So progressive increase in variance with spacing may be taken as evidence of non-homogeneous vegetation, and usually of a non-homogeneous environment.

It is interesting to compare the present results with those of Smith (1937), who was concerned with the very different problem of the variance of wheat yields in plots of different size. He found the logarithm of the variance was linearly related to the logarithm of plot size. Mean spacing would here be in general an increasing, but irregular, function of plot size, and it is not possible to convert from one variable to the other. However, Smith's results clearly reflect a continuous increase in environmental diversity with wider spacing between samples, and thus serve to support the present thesis.

Although the curves fitted have in each case been polynomial regressions of log cover on log spacing, there is no *a priori* reason to expect a relation of this particular form. The linear regression which appears satisfactory in the majority of instances is merely a first approximation, and that there are deviations from it by no means necessarily invalidates the general principle propounded of a continuous increase in variance with spacing. But some of the observed deviations seem to fit better with the hypothesis of stepwise increase adopted by Greig-Smith, and based rather on a homogeneous vegetation with a mosaic pattern. Greig-Smith and his students have tried to interpret all their data in terms of this model, drawing attention to one, two, or three "steps" in each curve. But the only statistical tests applied were of the significance of the difference between each successive pair of variances and, as Thompson has pointed out, the validity of the "stepwise" interpretation was often open to doubt.

Kershaw (1958, 1959) claims that most of his sets of data show three "peaks" and, while admitting that these "peaks" have not been demonstrated to be statistically significant, lays some stress on the argument that the approximate agreement between different sets of data in the position of these peaks provides mutual confirmation. Thompson (1958) uses a similar argument in discussing Phillips's (1954) results. But it is, to say the least, doubtful whether the data are sufficient to support this argument. If by a "peak" we understand a positive deviation from a smooth curve, random scatter of eight points about a curve will very frequently lead to three peaks. If we take Kershaw's subjective assessment of peaks (Kershaw 1958, Table 2; Kershaw 1959, Tables 2, 3, and 6), and compare the numbers recognized in the different sets of data with the numbers expected on random distribution of points about a curve, the results are as follows:

No. of "peaks"	No. of sets of data	
	Observed	Expected
4	4	3·0
3	35	28·4
2	41	42·7
1	6	11·9

$$\chi^2 = 4{\cdot}86$$

representing a satisfactory measure of agreement. In other words, the number of "peaks" recognized does not differ from those which might be expected had the relationship been correctly described by a smooth curve. It would seem that the number of variance estimates (7, if the between-transect variance is ignored) is hardly enough to enable three "peaks" to be identified with certainty, even if they exist. If we turn to the position of the "peaks", we notice that Kershaw regards "peaks" in different data at adjacent spacings (say, 1·6 and 3·2 m) as equivalent. Once the existence of the "peaks" is accepted, this is biologically very reasonable; but it is doubtful whether "peaks" at different spacings can properly be regarded as providing mutual confirmation when the number of spacings studied is so small. The change in position of the peak for *Agrostis tenuis* with sward maturity (equated with cover by this same species), shown in Table 2 of Kershaw (1958), is rather more convincing, though no such trend is evident in the other species.

A continuous increase in variance with spacing is not compatible with the simple idea of vegetation as a regularly repeating mosaic, but could certainly be generated by a system of superimposed mosaics subject to a good deal of random variation—which is a more realistic picture of vegetational possibilities. A clear-cut mosaic pattern would necessarily lead to a clear-cut "step" or "plateau" in the curve, but mosaic patterns at two scales superimposed, and perhaps combined with a general trend in composition across the area studied, might well give a curve with a virtually continuous increase in variance and no apparent "steps". But, however the continuous increase is generated, it clearly takes away from the idea of "minimal area" any objective significance which might have been ascribed to it.

It is clear, too, that the hypothesis of continuous increase in variance is inconsistent with the concept of a homogeneous plant community, but fully in accord with that of vegetational continua. Elsewhere (Goodall 1954*c*) doubt has been cast on the real existence of homogeneous areas of vegetation, and much of the evidence presented here serves to support these doubts—particularly the data from the Victorian Mallee for separate communities, in which the *a priori* expectation of homogeneity would be strongest. If one approaches the vegetation without preconceptions, however, the concept of continuous increase in variability is simpler and makes less demands on belief than that of distinct homogeneous areas. This is reflected in the simpler mathematical form, a linear regression of variance on spacing requiring only two parameters while the simplest form of the "stepwise" hypothesis calls for three. Using William of Occam's famous principle, the former hypothesis is accordingly to be preferred.

It is not suggested that a linear regression is always adequate to account for the increase of variance with spacing; some of the data here presented suffice to give the lie to such a suggestion. But it would seem to be a good first approximation on which complications can be superimposed where occasion demands.

It is noteworthy that the different species within the same area do not behave alike in this respect. Sometimes the regression is significant for certain species and not for others. Sometimes certain species show deviations from linearity suggesting a "step" where others do not. Where "steps" are suggested for more than one species, they usually are not indicated at the same spacing. And the regression coefficients for different species in the same vegetation vary widely. These differences suggest that the vegetation is not to be regarded as a well-knit community, but rather as a congeries of species, each a law to itself, some dependent on others in their distribution, some groups in common dependence on the same environmental factors—a view heterodox in synecology, but supported from time to time by a number of distinguished ecologists (Gleason 1917; Kylin 1926; Schmid 1950).

Although in principle the concept of vegetational continua is difficult to reconcile with the classification of plant communities, this conflict need not be of any importance in practice. Even if vegetation is varying continuously, it may well be convenient to single out particular types which can be taken as representative of the regions of the continuum around them. These types (the "noda" of Poore (1955)) can then be used very satisfactorily for descriptive purposes, while it is constantly borne in mind that they are no more than types—not classes into which all vegetation must willy nilly be fitted, and between which sharp boundaries are to be expected.

IV. Acknowledgments

This work was initiated, and the field observations were performed, while I was on the staff of the University of Melbourne Botany School; I should like to express my appreciation to Professor J. S. Turner, both for the facilities made available there and for his very helpful criticism of this paper in draft.

It is also a pleasure to express thanks to my wife for help in collecting the Rhyll data; to Mr. P. B. Leach and Mr. D. Leach of Walpeup for assistance in the Mallee; and to Mr. H. C. Dawkins for making the Uganda data available.

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Appendix I

By F. E. Binet*

In the main paper Dr. Goodall sets up a model specifying a linear or curvilinear relationship between the logarithm of the population variance of a conditional random variable and the logarithm of the conditioning variate. In the conventional notation of statistics this specification can be expressed as

$$\eta_x = \alpha + \sum_{l=1}^{l=r} \beta_l . x^l, \tag{1}$$

where

$$\left.\begin{aligned} \eta_x &= \log_{10}\sigma^2_{Z|X} \\ \text{and} \\ \sigma^2_{Z|X} &= V\{Z|X = x\}. \end{aligned}\right\} \tag{2}$$

In the two definitions of which (2) consists, Z is taken, in first approximation, to be "normally" distributed (i.e. according to the Gaussian distribution), and the conditioning variate is taken as $X = \log_{10}U$ (U being the measure, as defined in the main article, of the spacing between the units of the ecological observations; X being regarded, for the purposes of the specification in (1), as having the fixed value x). Consideration must be given to the fact that, although $s^2_{z|x}$ is an unbiased estimator of $\sigma^2_{Z|X}$, $\log_{10}s^2_{z|x}$ is not an unbiased estimator of $\log_{10}\sigma^2_{Z|X}$. The consequence of that bias is that the statistical parameters essential in the specification (1) cannot be estimated by the conventional statistical techniques from the usual estimates (i.e. $\log_{10}s^2_{z|x}$) of the right-hand side of (1), notwithstanding that they are "sufficient" (i.e. from the mathematical point of view the best possible) estimates.

This obstacle is, however, easily by-passed by applying corrections to the biased estimators $\log_{10}s^2_{z|x}$: these corrections are obtained in one elementary step from the entries in the second column of Table 1 of Bartlett and Kendall (1946). This paper explains fully the grounds of the bias (viz. s^2 is, in effect, σ^2 multiplied by a random variable, with expectation unity; thus, unbiased. On the other hand, $\log s^2$ is the sum of $\log \sigma^2$ and the logarithm of the same random variable: this logarithm is, of course, itself a random variable, but its expectation is *not* zero: this is the source of the bias), and gives a method for its elimination (the method is derived by expanding the cumulant-generating function of the random variable causing the bias: the logarithm of χ^2_ν/ν).

The following procedure, based on Bartlett and Kendall, is applied. Denoting by x_j the logarithm of the jth "spacing measure", by ν_j the degrees of freedom in the

* Poultry Research Centre, Division of Animal Genetics, C.S.I.R.O., Werribee, Vic.

s^2 belonging to that jth spacing, by s_j^2 just *that* $s_{\nu_j}^2$, and—following the specification (1)—by η_j the $\log_{10}$ of that σ_j^2 which is the expected value of s_j^2, an unbiased estimator of η_j can be constructed, viz.

$$\log_{10} s_j^2 + 0{\cdot}4343\alpha_{\nu_j} = \bar{y}_j \text{ (say)}. \tag{3}$$

Here, on the left-hand side, $0{\cdot}4343 = \log_{10} e$, where e is the base of the Naperian logarithms; while α_{ν_j} are the entries with positive sign of the second column of Bartlett and Kendall's Table 1—provided one equates the n in their first column with the above-defined ν_j. These values are shown there up to and including $n \leqslant 20$. Inspection of the second and third columns demonstrates, however, that

$$\alpha = \nu^{-1}(1 + \tfrac{1}{3}\nu^{-1}) \tag{4}$$

is an approximation, satisfactory for $\nu \geqslant 13$, to the accuracy required in practically all ecological projects. The estimator $\bar{y}_j$, on the right-hand side, can be taken as a realization of a modified random variable ($\bar{Y}_j$, say), satisfying

$$E\{\bar{Y}_j\} = \eta_j. \tag{5}$$

Next, it is noted that the correction term $0{\cdot}4343\alpha_{\nu}$ contains no random elements: consequently Bartlett and Kendall's findings with respect to the nearly normal distribution of $\log s^2$ (cf. columns 6 and 7 of their Table 1, and their Fig. 4) are applicable to Y; it is in view of that circumstance that (5) forms the basis of the application of the usual statistical routine for the computation of a and b, where $a = \hat{\alpha}$, $b = \hat{\beta}$, the respective estimates of the parameters α and β, completing the specification (1).

This routine was initially established by Gauss and Markoff (see, for instance, David and Neyman 1938). Its up-to-date versions are, essentially, weighted least-squares methods for fitting regression curves; this principle of weighted least squares is known to lead to maximum likelihood estimates, whenever the random variable, which is *not* taken as fixed, follows a *conditional* Gaussian distribution; and even when merely rather weak regularity conditions are satisfied, it leads to minimum-variance unbiased linear estimates. A working description of this routine, which can be applied reasonably easily, is contained in Anderson and Bancroft (1952, Chap. 14); to apply it to data transformed and adjusted in Bartlett and Kendall's manner, the parameters $\text{var}\{\bar{Y}_j\}$ are needed for all j. Referring to Bartlett and Kendall's Table 1, it can be found (by recalling that $0{\cdot}4343\alpha_{\nu_j}$ is free of random elements) that

$$\text{var}\{\bar{Y}_j\} = (\log_{10} e)^2 \,.\, \text{var}\{\log_e s_{\nu_j}^2\}$$

and hence that

$$\text{var}\{\bar{Y}_j\} = 0{\cdot}1886\beta_{\nu_j}^{-1}, \tag{6}$$

(where $0{\cdot}1886 = 0{\cdot}4343^2$, occurring in an analogous manner to $0{\cdot}4343$ in (3)), β_{ν_j}

being the entries of the fourth column of the Table 1 quoted above—taking again, analogously to the procedure for the α_{ν_j} values, the first column's n to be equal to our ν_j. For $n \geqslant 13$ one may, in analogy to (4), take

$$\underset{\nu}{\beta} = \frac{\nu-1}{2} \tag{7}$$

in lieu of the tabulated values, which extend to $\nu \leqslant 20$.

To simplify the weighting procedure, one may apply the routine (described by Anderson and Bancroft 1952) to *fictitiously* re-arranged data, as follows: one pretends that each $\bar{y}$ is the mean of N_j *fictitious* observations (of y_{j,m_j}; $m_j = 1, 2, \ldots N_j$), all of which are identically distributed, in Gaussian distribution, with means

$$\eta_j = \alpha + \sum_{l=1}^{l=r} \underset{l}{\beta} x_j^l$$

and equal variances; the value of that variance, v, is arbitrary, except that the N_j must be chosen so that

$$\frac{v}{N_j} = \text{var}\{\bar{Y}_j\} \text{ for all } j;$$

hence v must be chosen so that even the smallest $N_j \geqslant 2$. For the calculation of a confidence belt (or a tolerance belt) around the fitted regression line it is consistent with this fictional rearrangement to pretend that each fictional array yielded the same value of s^2, viz.

$$s_j^2 = v;$$

hence, that the residual sum of squares around the regression line is

$$v. \sum_j (N_j - 1).$$

SYSTEMATIC STUDIES OF THE ANATOMY OF THE GENUS *ACROTRICHE* R.BR.

I. THE LEAF

By Betsy R. Paterson*

[*Manuscript received July* 12, 1961]

Summary

The leaves of the 12 species of *Acrotriche* (Epacridaceae) have been examined anatomically from a systematic standpoint. It has been possible to distinguish between the species on the basis of leaf characteristics alone. These distinctions have been outlined in the form of a key to the species, and discussed fully in individual specific descriptions. The most valuable diagnostic characters are: presence or absence of papillose differentiation; shape of the leaf; shape of the sheath of fibres supporting the vascular bundle; presence or absence of a parenchymatous sheath around the bundles; and the presence or absence of calcium oxalate crystals and large fat globules. The specific delimitations based on the leaf anatomy agree with the morphological boundaries.

I. Introduction

This study, of the general leaf anatomy of *Acrotriche*, was made in connexion with a taxonomic study of the genus (Paterson 1960). The anatomy has been viewed from a systematic rather than a developmental standpoint.

Although the leaves of several species have been examined with respect to the family as a whole, no detailed descriptions of the specific similarities or differences within the genus have been made. The first worker was Simon (1891), who was concerned with the leaf structure of both the Ericaceae and the Epacridaceae, whilst Baccarini (1902), Solereder (1908), Étienne (1909), and more recently Copeland (1954) surveyed the leaf anatomy of the Epacridaceae as a whole.

This paper was undertaken in an attempt to determine the extent of specific variations, if any.

II. Material and Method

Material of *A. divaricata* was collected by A. McCusker and that of *A. depressa* by Hj. Eichler. The remainder of the material was collected by the author on various field trips. In the four species where it was not possible to collect fresh material, portions of herbarium specimens were used. This material was boiled with water, and then treated in the same manner as the fresh specimens.

All material was fixed in formyl–acetic–alcohol. Some material was dehydrated by the ethyl alcohol–xylene series, but better penetration was obtained by a modification of Hyland's (1941) n-butyl alcohol series. The material was then embedded in paraffin. Where softening was necessary, a modification of Gifford's (1950) schedule as described by Reeve (1954) was used. This method was most

* Department of Botany, University of New England, Armidale, N.S.W.

satisfactory, causing no apparent distortion of tissues. Although some hand sections were made, most of the material was cut on a microtome between 10 μ and 15 μ. The most satisfactory stain was a combination of safranin with a mixture of fast and light green in clove oil. Epidermal strips were stained in a saturated alcoholic solution of Sudan IV.

Throughout this paper, the term "central region" has been used to refer to the thin area of the outer epidermal wall above the protoplast as seen in surface view.

All drawings were made with a camera lucida; in some cases details from more than one section were added to make a composite drawing.

III. Results

(*a*) *General Leaf Features*

The leaf of *Acrotriche* is xeromorphic, possessing a thick cuticle and heavily thickened epidermal cells. In addition there is a large mass of sclerenchyma associated with each vein. In most leaves this group of fibres is crescent-shaped (Figs. 2–5, 7–12) and supports the vascular bundle on the abaxial side. In *A. divaricata* (Fig. 1) and *A. aggregata* (Fig. 6), each bundle is completely surrounded by fibres.

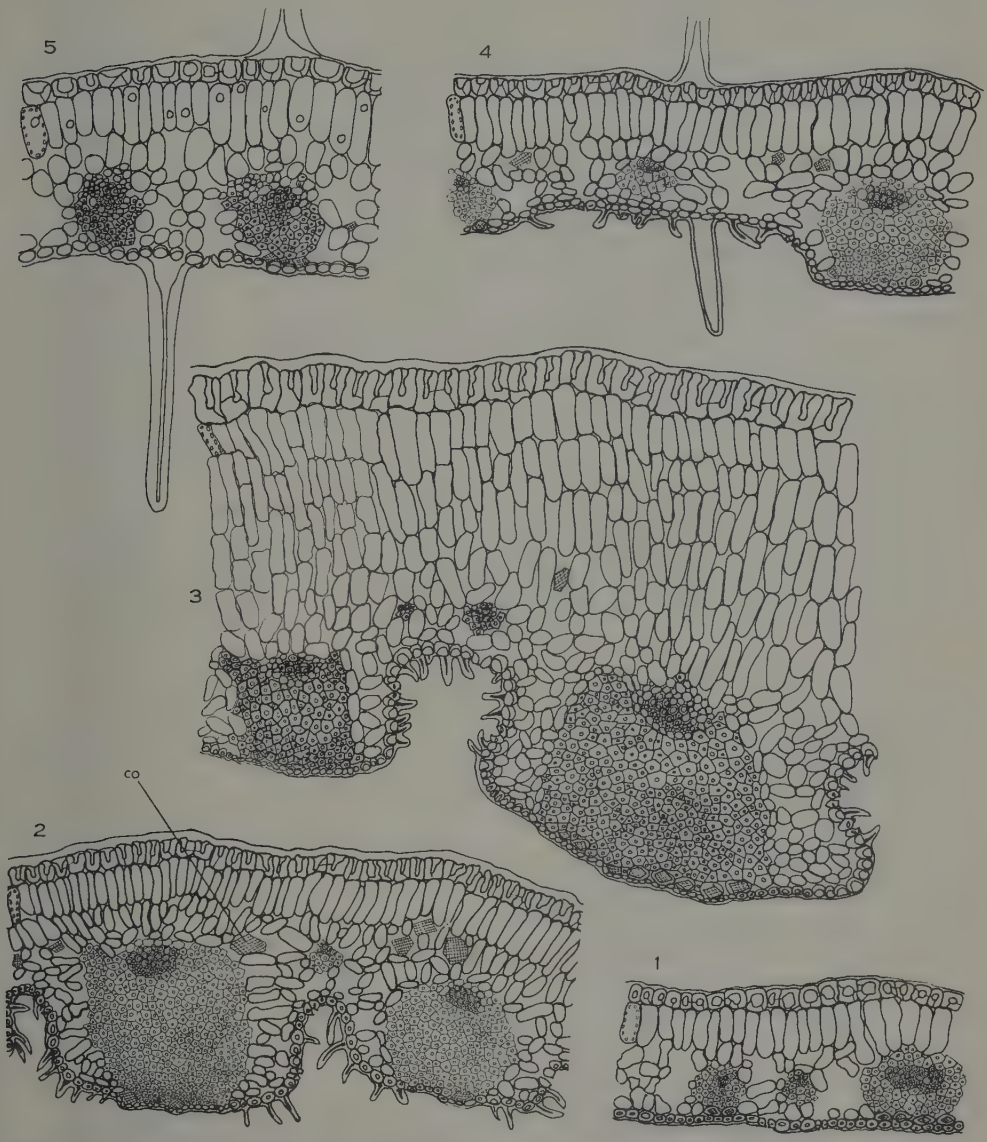
The petioles (Figs. 13, 14) are short and usually semi-elliptical in transection. The epidermal cells are covered by a thick cuticle. The cells of the cortex have a tendency to be collenchymatous towards the epidermis, becoming parenchymatous towards the centre. Numerous crystals of calcium oxalate are found in the cortex. The vascular elements occupy the centre of the petiole and initially only one bundle is present but this later divides to three. These bundles are completely surrounded by fibres (Fig. 14) and it is only when the bundle enters the lamina that the fibres take up the positions typically found there. The thick-walled fibres contain numerous pits. The quantity of sclerenchyma accompanying each vein varies with its importance, so that a minor vein may have almost none.

The few phloem elements are on the abaxial side of the bundle and consist of small sieve tubes and companion cells with a lot of phloem parenchyma. The xylem is mainly composed of thick-walled vessels with small lumina, and the walls usually have spiral or reticulate thickening. In addition, there are a few tracheids and some parenchyma.

The shape and thickness of the lamina vary with the species. The leaves are all palmately veined, the central veins are approximately parallel and the lateral veins spread towards the margins with numerous irregular anastomoses. The end of each vein usually consists of a single vessel without any associated phloem or sclerenchyma.

In all species the epidermal cells are covered by a thick cuticle. The cells are usually thickened on the three inner walls although in some species, e.g. *A. aggregata*, all the walls are involved. In others, such as *A. rigida* (Fig. 2), the adaxial epidermal cells are thickened on three walls, while the abaxial ones are more or less rounded and uniformly thickened. Numerous pores occur in the thickened walls of all epidermal cells, and the shape of the lower cells varies with position; those between the veins are generally wider, and more irregular than those below.

The stomates, which are found only on the abaxial surface, have their longitudinal axes parallel with the veins and they are usually slightly raised above the surrounding epidermal cells (Figs. 15, 16). A small papilla is associated with each



Figs. 1–5.—Transections of leaves. ×130. Fig. 1.—*A. divaricata*. Fig. 2.—*A. rigida*. Fig. 3.—*A. affinis*. Fig. 4.—*A. serrulata*. Fig. 5.—*A. fasciculiflora*. *co*, Calcium oxalate crystals.

guard cell near the outer orifice, but no ridges were seen near the inner orifice, as reported by Baccarini (1902) for certain members of the Epacridaceae.

Hairs or papillae, which are differentiated by the epidermal cells, are unicellular. Large mature hairs may spread over three or more epidermal cells, whereas

papillae usually only extend over one cell or part thereof. The cell walls of long hairs have relatively uniform thickening. This contrasts with the papillae, and the cells forming the scabrous margins, which are thickened towards the base resulting in a conical appearance.

The well-developed palisade mesophyll is rich in chloroplasts. Where more than one layer of palisade exists, the cells forming the first layer are longer and often slightly wider than in the succeeding layers. In several species, e.g. *A. cordata* and *A. halmaturina*, a large fat globule is present in most cells of the first palisade layer, though similar globules are occasionally found in succeeding layers and in scattered spongy mesophyll cells. Normally only one globule is present per cell. The amount of spongy mesophyll varies with the thickness of the leaf. Calcium oxalate crystals are frequently scattered in the intercellular air spaces. The structure of the vascular bundles is the same as described for the petiole but in some species, such as *A. prostrata* (Fig. 7), there is a parenchymatous sheath around the bundle.

Between the fibres supporting the major bundles and the lower epidermis, there is a row of cells which Étienne (1919) termed an "endodermis", "facilement identifiable par suite de la quantité très considérable de cristaux qu'elle présente à la partie dorsale des faisceaux". These cells are thin-walled, and larger than the neighbouring fibres. For convenience, this row of cells will be termed an "endodermis" or "endodermal cells" throughout this paper. In all species crystals of calcium oxalate are present in these cells, and are frequently twin crystals. These crystals are visible through the cells of the abaxial epidermis (Fig. 17) with the exception of *A. cordata*. In this species (Fig. 20) the epidermal cells are heavily thickened.

(*b*) *Key to the Species of* Acrotriche *based on Leaf Characteristics*

A. Leaves deeply grooved
  B. Papillae adjacent to veins as well as in grooves ..... 5. *rigida*
  BB. Papillae in grooves only ..... 4. *affinis*
AA. Leaves smooth or with shallow grooves
  C. Leaves with recurved margins
    D. Leaves without hairs or papillae ..... 10. *ramiflora*
    DD. Leaves with hairs or papillae
      E. Leaves lanceolate or broadly lanceolate
        F. Fat globules present in palisade tissue
          G. Long hairs and papillae present ..... 11. *halmaturina*
          GG. Long hairs only present ..... 12. *fasciculiflora*
        FF. No fat globules present in palisade tissue ..... 6. *prostrata*
      EE. Leaves linear-lanceolate or subcordate
        H. Leaves linear-lanceolate ..... 3. *serrulata*
        HH. Leaves subcordate, reflexed ..... 9. *depressa*
  CC. Leaves without recurved margins
    J. No calcium oxalate crystals present in the "endodermis" when viewed externally ..... 8. *cordata*
    JJ. Calcium oxalate crystals present in the "endodermis" when viewed externally
      K. Fibre sheath crescent-shaped ..... 7. *patula*
      KK. Fibre sheath almost completely surrounding the bundles
        L. Numerous small papillae on lower epidermis. Leaves usually greater than 12 mm long ..... 2. *aggregata*
        LL. Papillae, if any, very small. Leaves usually under 12 mm long ..... 1. *divaricata*

(*c*) *Individual Specific Descriptions*

1. **A. divaricata** R.Br. (Fig. 1). Morphologically the leaves are lanceolate and flat, with pungent tips. Both upper and lower surfaces are glabrous, or the lower surface may bear very small protuberances or papillae.

Average thickness of the leaf is 0·125–0·156 mm. The cuticle on the adaxial surface is approximately twice as thick as that on the abaxial. The upper epidermal cells are angular in cross section with uniformly thickened walls. In surface view the cells are elongate with thick, wavy walls forming ridges; the surface between the ridges is rough.

There are numerous plastids in the single row of palisade cells. The minor veins consist of several vessels and a few phloem elements bounded by a parenchymatous sheath, and several scattered fibres. In contrast, the major veins, while showing little increase in vascular elements, each have a well-defined mechanical sheath of fibres completely surrounding the bundle. Although there are only about two rows of fibres on the adaxial side, on the abaxial they extend to the "endodermis".

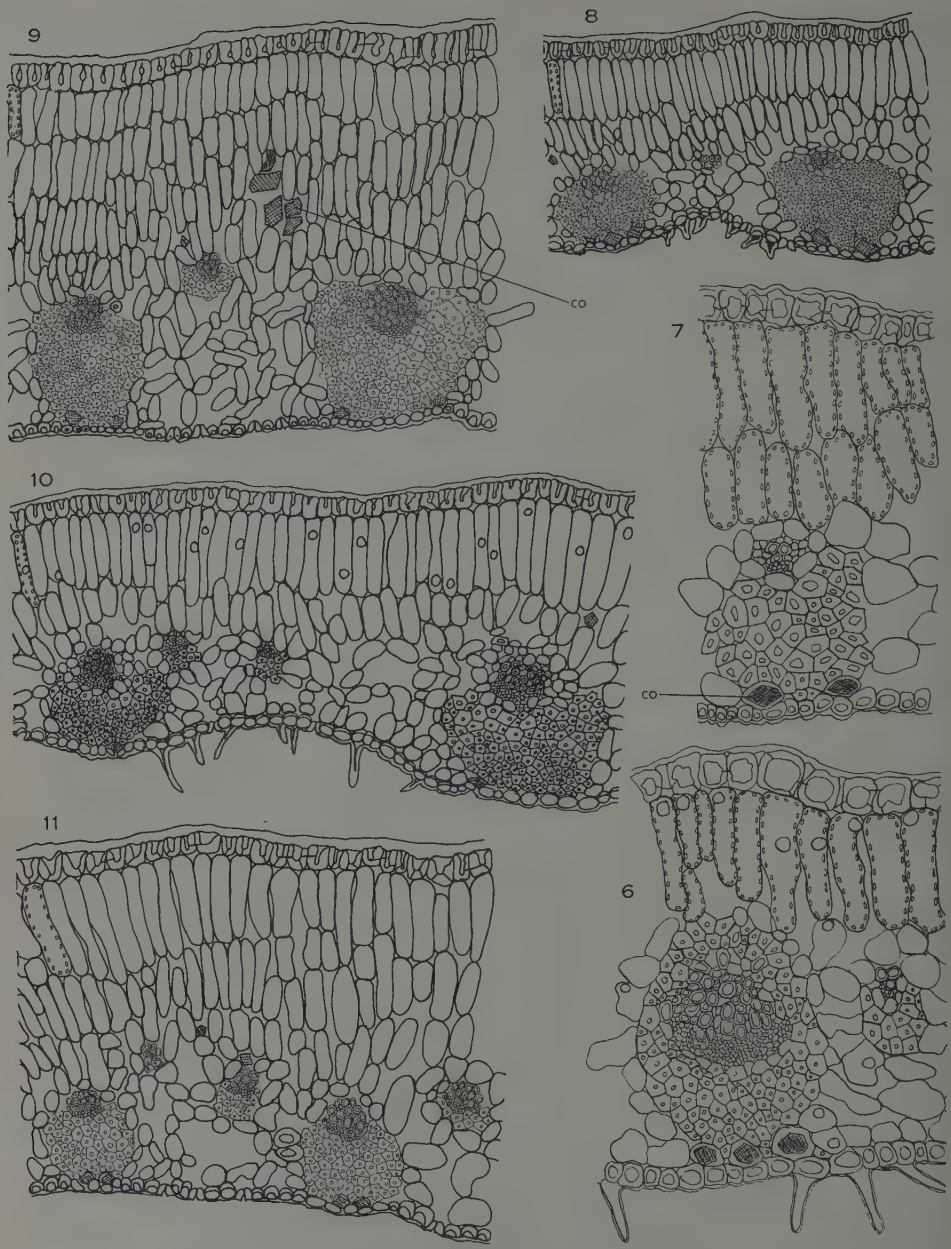
The lower epidermal cells are rounded, with uniformly thickened walls and a small lumen. In surface view the shape varies; the cells below and closely adjacent to the veins are elongated with wavy walls, whereas the intervening cells are wider with stomates scattered amongst them.

2. **A. aggregata** R.Br. (Figs. 6, 16). The leaves are lanceolate, flat and spreading, with a pungent, mucronate tip. Although the adaxial surface is glabrous, the abaxial bears numerous small microscopic hairs. These give the undersurface of the leaf a whitish appearance.

A transection of a mature leaf shows an average thickness of 0·24–0·26 mm; the upper cuticle is approximately twice the thickness of the lower cuticle. The greatest concentration of the papillae, which completely cover the abaxial surface, is between the veins. The length of the papillae varies from 0·015 to 0·625 mm. Adaxial and abaxial epidermal cells are similar in form, and although the former are comparatively larger, both are uniformly thickened on all four walls. In surface view the ridges formed by the projection of the thickened side walls and middle lamellae are wavy, and the thickening is irregular. The central region, between the ridges of these somewhat rectangular cells, is smooth. The abaxial cells are much broader, and the walls are thinner in surface view, than those of the adaxial surface. The cells beneath the veins are slightly narrower, and more elongate than the others. The small papillae on the stomates (Fig. 16) are more prominent than in the preceding species.

There are three to four rows of fibres present on the adaxial side of the major bundles. The xylem and phloem elements are well defined, the latter occupying a crescent-shaped zone on the abaxial side of the xylem.

The palisade tissue consists of one or two rows; the second row, when present, is half the length of the first. Most of these palisade cells, as well as a few spongy mesophyll cells, contain fat globules. No intercellular crystals of calcium oxalate are present, although crystals are present in the "endodermal cells".



Figs. 6–11.—Transections of leaves. Fig. 6.—*A. aggregata*. ×220. Fig. 7.—*A. prostrata*. ×220. Fig. 8.—*A. depressa*. ×130. Fig. 9.—*A. patula*. ×130. Fig. 10.—*A. halmaturina*. ×130. Fig. 11.—*A. ramiflora*. ×130. *co*, Calcium oxalate crystals.

3. **A. serrulata** (Labill.) R.Br. (Fig. 4). This species has linear-lanceolate leaves with mucronate, pungent tips. The margins are serrated and ciliate, with long scattered hairs on both surfaces. Those on the abaxial surface are associated with very small hairs or papillae, which occur in the shallow grooves between the veins.

The average thickness of a mature leaf in transection is 0·275–0·3 mm, with the upper cuticle twice as thick as the lower. The papillae vary in length from 0·01 to 0·0175 mm. The adaxial epidermis consists of cells which are greatly thickened on their inner walls compared to the outer wall. Seen from above these cells are elongate and the wavy walls leave a very narrow central region, which has a rough uneven surface resembling cracked mud. The stomates and papillae are associated with the abaxial epidermal cells, which are rounded and comparatively uniformly thickened. In surface view these cells are elongate and thin-walled, whilst the intervening cells are rectangular with wavy walls.

The vascular bundle occupies a relatively small area in these leaves. The number of vascular elements is much reduced; they are surrounded by a parenchymatous sheath, and on the abaxial side the fibres form a crescent-shaped mass. In the palisade the longest cells are in the first row, while those in the succeeding one or two rows are shorter and slightly elongated. Crystals of calcium oxalate are scattered throughout the mesophyll, mainly associated with the spongy tissue.

4. **A. affinis** DC. (Fig. 3). The leaves look fairly similar to those of the previous species, but they are broader and thicker, and quite rigid compared to *A. serrulata*. The margins are slightly serrated. The adaxial surface is glabrous, while the abaxial is deeply grooved and bears numerous microscopic hairs, together with occasional long hairs in the grooves. The thickness of the leaf varies from 0·51 to 0·69 mm.

5. **A. rigida** B. Paterson (Fig. 2). The linear-lanceolate, rigid leaves spread at right angles to the stem with the margins much recurved. The adaxial surface is scabrous, whilst the lower has numerous papillae arising in the deep grooves and also adjacent to the veins. These papillae vary in length from 0·01 mm for those near the veins to 0·025 mm for those in the grooves.

The average thickness of the leaves varies from 0·275 to 0·3 mm, with both cuticles of approximately equal thickness. The adaxial epidermal cells have a thin outer wall, but the other walls are greatly thickened. In surface view these cells are long and thin with a very narrow, slightly roughened, central region. The abaxial cells are small with very thick walls. Externally these cells are rectangular, and the outer wall has rough areas irregularly distributed. Below the veins the cells are smaller and more elongated than are those between. Stomates only occur towards the bases of the grooves.

The palisade tissue is one to two cells thick, with the second row much shorter than the first. A few fat globules may occur in these cells. The small vascular bundle is enclosed by a parenchymatous sheath. Calcium oxalate crystals are found in the "endodermis", and also scattered amongst the spongy mesophyll tissue with an odd crystal wedged amongst the palisade.

6. **A. prostrata** F. Muell. (Figs. 7, 15). The thin leaves vary from narrow to broadly lanceolate; they are ciliate with recurved margins and a pungent tip. The average thickness of a mature leaf varies from 0·262 to 0·287 mm, and the cuticle on both surfaces is approximately equal in thickness. Long hairs occur on both surfaces with the greatest concentration on the marginal areas.

The adaxial epidermal cells are uniformly thickened with a small lumen, circular in cross section. In surface view these cells have a wide smooth central region. The cells of the abaxial epidermis are thickened on all walls but the amount of thickening is less in those below the veins. On the surface the cells below the veins are elongate, whilst the others are somewhat rectangular and thin-walled, with numerous stomates and papillae.

There are two layers of palisade cells, the second shorter than the first. The spongy mesophyll cells are fairly closely packed, and a very distinct parenchymatous sheath surrounds the vascular elements. The fibres form a crescent-shaped group below the main vascular bundles, though a few fibres may encircle the minor bundles. The lumina of these fibres are uniformly wider than in any other species. No calcium oxalate crystals were found except in the "endodermis".

7. **A. patula** R.Br. (Figs. 9, 18). Rigid, ovate-lanceolate leaves, concave above, with the margins slightly serrated towards the apex, are characteristic of this species. The adaxial surface of the leaf is glabrous and shiny, while the abaxial is discoloured.

The thickness of the mature leaf varies from 0·425 to 0·45 mm. The cuticle is thick, 0·005–0·0075 mm, and both cuticles have approximately the same thickness. The cells of the adaxial epidermis are elongate in cross section, with the inner walls considerably thickened, and the lumen is long and narrow. In surface view the cells are elongate, with thick wavy walls and a very narrow central region, which in some places is almost eclipsed. The abaxial cells are again in two forms: below the veins they are thin-walled and rectangular; the remainder, amongst which the stomates occur, are shorter and broader than the former and their inner walls are thickened.

The palisade tissue is composed of three to four rows of closely packed cells. The spongy mesophyll cells are relatively closely packed and they have a tendency to be narrower and more elongate than in any other species. The vascular bundle is reasonably well developed, consisting chiefly of xylem vessels with some parenchyma and a small quantity of phloem. Numerous calcium oxalate crystals are scattered throughout the mesophyll tissues as well as in the "endodermis". A few sclereids are often found.

8. **A. cordata** (Labill.) R.Br. (Figs. 12–14, 17). The characteristic feature of the leaves of this species is the blunt callous tip. The leaves vary from ovate to oblong, the upper surface being glabrous while the lower has small protuberances between the veins.

The thickness of the leaf varies from 0·475 to 0·5 mm, with the adaxial cuticle 0·0125 mm thick and the abaxial 0·005 mm. The adaxial epidermal cells are greatly thickened except for the outer wall. In surface view these cells are

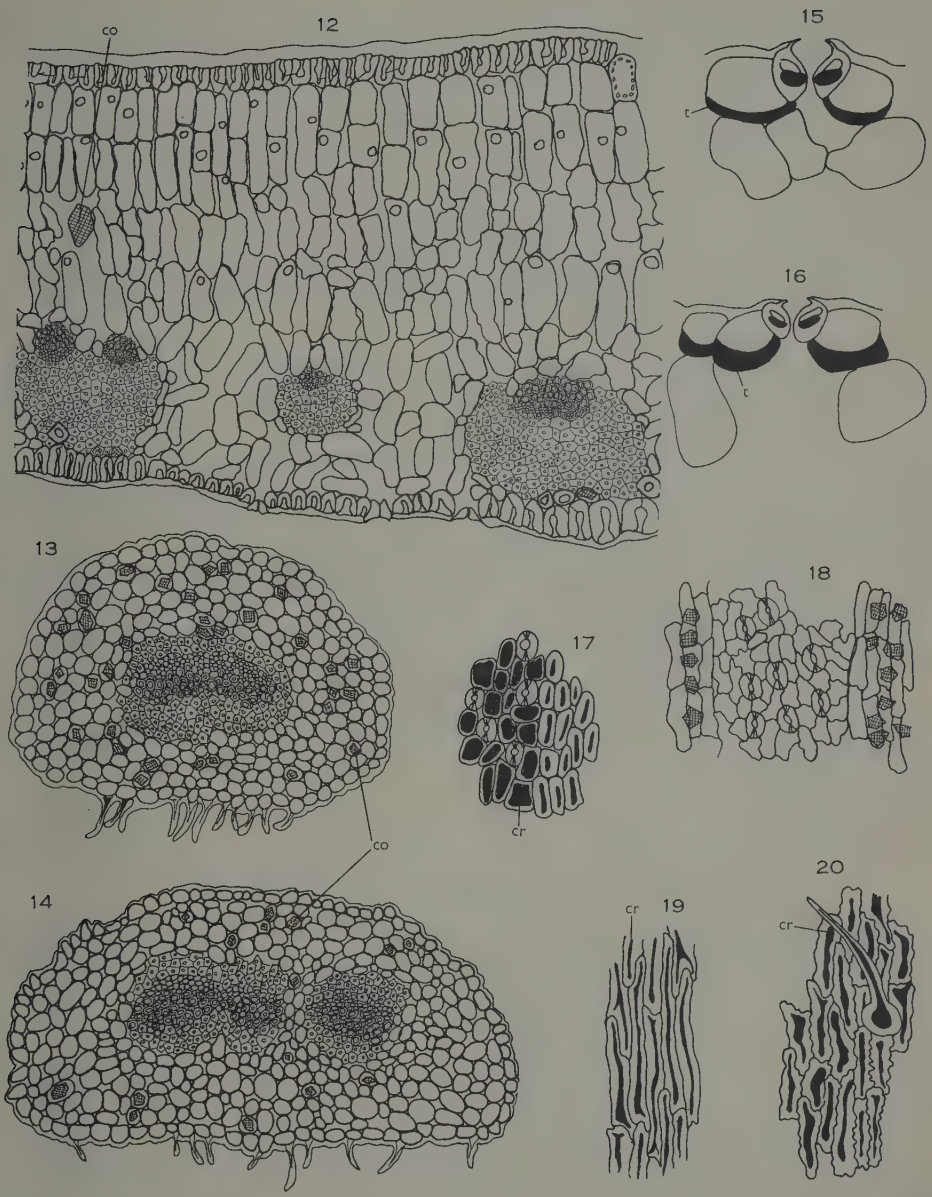


Fig. 12.—Transection of leaf of *A. cordata*. ×130. Figs. 13–14.—Transections of petiole of *A. cordata*. ×130. Fig. 13.—Base of petiole. Fig. 14.—Petiole at base of lamina. Fig. 15.—Stomate of *A. prostrata*. ×550. Fig. 16.—Stomate of *A. aggregata*. ×550. Fig. 17.—Surface view of abaxial epidermis of *A. cordata*. ×130. Fig. 18.—Surface view of abaxial epidermis of *A. patula*. ×130. Fig. 19.—Surface view of adaxial epidermis of *A. depressa*. ×130. Fig. 20.—Surface view of adaxial epidermis of *A. halmaturina*. ×130. *co*, Calcium oxalate crystals; *t*, thickening; *cr*, central region of the epidermal cells.

elongate with very thick wavy walls and a narrow slightly roughened central region. The cells of the abaxial epidermis are similar to those of the adaxial epidermis, but those beneath the veins are longer and have thicker walls than the intervening cells.

The mesophyll tissues consist of about four rows of palisade associated with a small amount of spongy tissue. Large fat globules occur in most of the palisade cells, predominantly in the first two rows. A few calcium oxalate crystals are found in the intercellular spaces as well as in the "endodermal cells". In the latter, the crystals cannot be seen externally owing to the thickening of the epidermal cells. Scattered sclereids are often associated with the fibres supporting the vascular bundle.

9. **A. depressa** R.Br. (Figs. 8, 19). The leaves, which are usually subcordate but may be ovate-lanceolate, have rough slightly recurved margins and a mucronate tip. The abaxial surface is discoloured by the numerous papillae which arise between the veins. Their height ranges between 0·0125 mm and 0·0175 mm. The mature leaf varies in thickness from 0·2 to 0·25 mm, and the adaxial cuticle is twice the depth of the abaxial. With the exception of the outer wall, all the adaxial epidermal walls are thickened. From above they appear elongate with thick ridges (Fig. 19) with a rough, narrow central region. The abaxial cells are rounded, and the walls are thin except for those between the veins, which are slightly thickened. The papillae are found between the veins.

There are usually two rows of palisade, followed by somewhat elongated spongy cells, and a parenchymatous sheath surrounds the few vascular elements. Calcium oxalate crystals are found in the "endodermis" and scattered amongst the spongy mesophyll.

10. **A. ramiflora** R.Br. (Fig. 11). The leaves are linear-lanceolate with pungent tips and recurved margins, which are smooth or slightly serrated. The adaxial surface is scabrous, and the abaxial bears small papillae between the veins.

The depth of an average leaf ranges from 0·35 to 0·375 mm, with the adaxial cuticle being four times as thick as the abaxial cuticle. The degree of thickening on the inner walls of the adaxial epidermal cells results in the formation of a very small lumen. It decreases towards the outer edge of the lateral walls, with the result that in surface view the walls appear much thinner than in any other species. These cells tend to be rectangular rather than elongated. The abaxial epidermal cells between the veins are heavily thickened on the inner walls, while those below are rounded and thin-walled. Externally, the former cells appear squarish with thin wavy walls while the latter are thin-walled, rectangular cells.

A fat globule is present in almost every cell of the two to three rows of palisade tissue, but there are none in the somewhat elongated spongy cells. The vascular tissue is surrounded by a parenchymatous sheath. Calcium oxalate crystals are scattered amongst the spongy mesophyll as well as in the "endodermis".

11. **A. halmaturina** B. Paterson (Figs. 10, 20). This species has lanceolate leaves with recurved margins. Long hairs are borne on both surfaces in addition to the papillae present on the lower epidermis.

A mature leaf averages 0·275–0·325 mm in thickness, with the adaxial cuticle twice as thick as the abaxial. The papillae vary in length from 0·03 to 0·05 mm whilst the hairs are considerably longer. The adaxial epidermal cells are thickened on their inner walls only; they appear rectangular (Fig. 20) in surface view, the central region of which is roughened. The abaxial cells are rounded, and thin-walled except between the veins when the inner wall is thickened. From above the cells below the veins are rectangular, whilst the intervening cells are broader and irregular in shape.

Fat globules are present in the first palisade row but not in the second. The comparatively large number of vascular elements are surrounded by a parenchymatous sheath. Scattered crystals of calcium oxalate are found in the spongy tissue and in the "endodermis".

12. **A. fasciculiflora** (Regel) Benth. (Fig. 5). Numerous long hairs cover both surfaces of the lanceolate leaves, their greatest concentration being on the recurved margins. Occasionally a few papillae are found on the abaxial surface between the veins.

This species has one of the thinnest leaves found in the genus, a mature leaf ranging between 0·137 and 0·225 mm. The adaxial cuticle is twice as thick as the abaxial. Thickening is sometimes present on the outer wall of the adaxial epidermis, but it is usually confined to the inner walls. In surface view these cells are elongate with wavy walls, and the smooth central region is approximately half the width of the cell. The abaxial epidermal cells are rounded, and thickened on the inner wall. The cells appear uniform except that those between the veins are slightly wider in surface view, though they are all rectangular.

Palisade tissue forms a single row in which nearly every cell contains a large fat globule. The spongy mesophyll cells are large and rounded. The few vascular elements are usually surrounded by a parenchymatous sheath but in some cases this may not enclose the bundle. Some calcium oxalate crystals are found.

IV. Discussion

This study demonstrates that specific anatomical variations occur in the leaves, and that these variations may be used to distinguish the species. This means that once a specimen is placed within the genus, it can be identified without reference to floral characters, although these assist confirmation. It is particularly useful for comparing two specimens, one complete and one vegetative. There appears to be no single leaf character peculiar to the genus, but this cannot be confirmed before a complete study of other genera is made.

Undoubtedly the most valuable diagnostic character is the presence or absence of papillose differentiation, together with their position and size. The other main features are:

(1) the shape of the leaf, e.g. lanceolate or subcordate;

(2) the shape of the sheath of mechanical fibres supporting the bundles, e.g. whether they surround the bundle as in *A. aggregata* or are only on one side as in *A. affinis*;

(3) the presence or absence of a parenchymatous sheath;

(4) the presence or absence of calcium oxalate crystals and large fat globules.

Within limits, species may be separated from one another by the depth of palisade, though here allowance must be made for the age of the leaf. The palisade of an old leaf is usually much longer and sometimes there is even an additional row compared with a young leaf of corresponding length and breadth. It is thus desirable to compare leaves of similar maturity. The nature of the thickening of the adaxial epidermal walls may be useful, as in *A. divaricata* (Fig. 1) and *A. serrulata* (Fig. 4), where in the former all walls but in the latter only the inner walls are thickened. Useful, but of minor importance, is the depth of the grooves between the veins. This is the most useful method of distinguishing between *A. serrulata* (Fig. 4) and *A. affinis* (Fig. 3).

The variations found in this study agree with the specific delimitations outlined in an earlier paper (Paterson 1960), and confirm that the morphological and anatomical features in *Acrotriche* are interlinked.

V. Acknowledgments

The author extends her sincere thanks to Dr. G. L. Davis for her guidance and supervision throughout this work. Appreciation is expressed to Miss A. McCusker and Dr. Hj. Eichler for assistance in collecting material.

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THE GENUS *OTTOCHLOA* DANDY (GRAMINEAE) IN AUSTRALIA AND ITS RELATIONSHIP TO *ICHNANTHUS OBLONGUS* HUGHES

By M. Lazarides*

[*Manuscript received June* 28, 1961]

Summary

A taxonomic account is given of the two Australian species of *Ottochloa*. One of the species, *O. nodosa* (Kunth) Dandy, has not been recorded previously for Australia as such. The taxa, *Ichnanthus oblongus* Hughes, unidentified in an earlier paper (Lazarides 1959), and *Panicum aequabile* Domin, are reduced to synonymy.

Introduction

In a previous study on the genus *Ichnanthus* Beauv. (Lazarides 1959†), three species of the Australian representatives were re-established in *Panicum*, one was transferred to the same genus, and one was retained in *Ichnanthus*. One further species, *Ichnanthus oblongus* Hughes, remained unplaced as type material had not been seen. Since then, information received from the Royal Botanic Gardens, Kew, (C. E. Hubbard, personal communication) confirms the fact that the type of Hughes's species, "Percy Island, margins of bushes, ? coll. A. Cunningham" agrees perfectly with Kew's material of *Ottochloa nodosa* (Kunth) Dandy. Dr. Hubbard states also that "the specimen of this species" (i.e. *Ottochloa arnottiana* (Nees ex Steud.) Dandy = *O. nodosa*) "collected at Yandina by F. M. Bailey on 1/3/1891" (Plate 1) "is a good match with the type of *Panicum aequabile* Domin" and has kindly given permission for the reductions to synonymy to be published.

The following is a brief account of the genus as it occurs in Australia.

Genus OTTOCHLOA Dandy

Ottochloa Dandy, J. Bot. **69**: 54 (1931).

Hemigymnia Stapf in Prain, Fl. Trop. Afr. **9**: 741 (1920) non Griff. (1843).

Spikelets dorsally compressed, falling entire from the pedicels, loosely appressed in narrow very short often distant racemes to the primary branches or (in the upper part) to the rhachis of a panicle. *Lower floret* reduced to the lemma, occasionally staminate and with a well-developed palea. *Upper floret* bisexual, as long as the spikelet, rugulose, dorsally flattened. *Glumes* and *lower lemma* thinly membranous, usually glabrous and smooth, narrowly hyaline on the margins; the *glumes* equal or subequal in length and much shorter than the spikelet; the *lower lemma* as long as the spikelet, usually with a shallow longitudinal depression on the back. *Upper lemma* firmly membranous, obtuse and mucronulate, obscurely 3–5-nerved; the

* Division of Land Research and Regional Survey, C.S.I.R.O., Canberra.

† Lazarides, M. (1959).—The Australian species referred to *Ichnanthus* Beauv. (Gramineae). *Aust. J. Bot.* **7**: 328–46.

margins incurved, ciliolate upwards, with narrowly hyaline edges. *Palea* as long as the lemma, thinly to firmly membranous, 2-keeled, depressed between the keels; the keels indurated, with incurved hyaline wings, and embraced for their full length by the lemma. *Perennials* with decumbent culms rooting from the lower nodes. *Blades* flat, thin. *Ligule* membranous, minute. *Panicle* open or contracted, the branches usually solitary or paired and distant, with few very short secondary branches; rhachis, branches, and pedicels very slender, scabrous or scaberulous; rhachis terete, becoming angled upwards; pedicels widened at the apex.

The genus is represented in Australia by two species, one of which is endemic. *Ottochloa nodosa* (Kunth) Dandy, on the other hand, is widely distributed in tropical Africa, India to Indo-China, Malaysia, and the Philippines. In Australia, the known generic range of distribution extends along the eastern coast of Queensland and New South Wales from Cooktown to Gosford with *O. nodosa* largely tropical and *O. gracillima* C. E. Hubbard mainly warm temperate in occurrence.

On examination of the 47 specimens available for study, the principal specific differences appear to rely on size rather than morphology. As shown later in the text, there are a small number of intermediate forms which are difficult to place in either species. With further collections and wider study, it may be found that the present species are merely climatic extremes of the same taxon.

Key to the Species

A. Spikelets usually 3–3·5 mm long; lateral pedicels 1·25–2·25 mm long. Panicles usually 7–15 cm long, with 4–12 primary branches. Blades usually 4·5–10 cm long. Plant rather stout, the flowering culms 30 cm or more high. Internodes rarely less than 3·5 cm long ..... *O. nodosa*

AA. Spikelets usually 2 mm long; lateral pedicels up to 1 mm long. Panicles usually 3–5 cm long with 2–4 primary branches. Blades usually less than 3·5 cm long. Plant very slender, the flowering culms often less than 15 cm high. Internodes mostly less than 2·5 cm long, rarely exceeding 3·75 cm ..... *O. gracillima*

Ottochloa nodosa (Kunth) Dandy, J. Bot. **69**: 55 (1931); *Panicum nodosum* Kunth, Rev. Gram. **1** (Suppl. 9) (1830) (not seen), Enum. Pl. **1**: 97 (1833); *Panicum arnottianum* Nees ex Steud., Syn. Pl. Glum. **1**: 59 (1854); *Panicum aequabile* Domin, Biblioth. Bot. **20** (85): 297 (1915); *Ichnanthus oblongus* Hughes, Kew Bull. **1923**: 328 (1923); *Ottochloa arnottiana* (Nees ex Steud.) Dandy, J. Bot. **69**: 55 (1931).

Types of Synonyms.—Holotype of *Ichnanthus oblongus*: Percy Island, *A. Cunningham* (not seen).

Holotype of Panicum aequabile.—In rain forest and *Pandanus* swamp, Waterfall Creek on Yarrabah Mission Station, Mission Bay, east of Cairns, *Domin* (not seen).

Decumbent perennial, the flowering culms 30 cm or more high. *Culms* simple or branched, with several nodes, prominently striate, glabrous or almost so, usually smooth. *Leaf sheaths* up to half as long as the internodes, prominently striate, tight or the lower becoming loose, hispid with tubercle-based spreading hairs or almost glabrous, tubercled or almost smooth, ciliate on one margin. *Ligule* 0·25 mm

Fig. 1.—*Ottochloa nodosa* (Kunth) Dandy from *Blake* 21208. *A*, plant. ×½. *B*, panicle branch. ×5. *C*, spikelet (front view). ×10. *D*, spikelet (back view). ×10. *E*, glumes. ×10. *F*, lower lemma. ×10. *G*, floret (front view). ×10. *H*, floret (back view). ×10.

long; orifice often sparsely bearded. *Blades* linear or linear-lanceolate, 2–15·5 cm long, up to 10·5 mm wide, abruptly constricted at the base, long attenuate and acute at the apex, with numerous prominent nerves and thickened white scabrous margins; the upper surface usually glabrous, scabrous or scaberulous on the nerves; the lower surface usually paler, glabrous or sparsely hispid with tubercle-based hairs, almost smooth, the mid vein prominent and white. *Panicle* usually open, 7–22 cm long, up to 4·5 cm wide; primary branches up to 12, the lower up to 13 cm long, and often with short secondary branches bearing 3–12 spikelets; lateral pedicels 1·25–2·25 mm long. *Spikelets* narrowly—to oblong—elliptic, obtuse, 2·5–4 mm long, 1 mm wide. *Glumes* ovate, subacute or obtuse; the lower from slightly less to slightly more than half as long as the spikelet, 3 (rarely 5)-nerved; the upper half to three-quarters as long as the spikelet, mostly 5-nerved. *Lower lemma* subacute to obtuse, narrowly elliptic, mostly 7-nerved. *Upper lemma* and palea elliptic-oblong, obtuse. (Fig. 1, Plates 1, 2.)

QUEENSLAND: Cook District: Kuranda, *White* 1553, 28.ii.1922 (BRI); Mossman River Gorge, *Brass* 2122, 9.ii.1932 (K, BRI); Daintree River, *Brass* 2200, 29.ii.1932 (K, BRI); Basilisk Range, South Johnstone, *Langdon* 5.iii.1938 (BRI); near Bundulla River bridge, Atherton, *Lazarides* 4230, 28.ii.1954 (BRI, NT, K, NSW, MEL, AD, PERTH, CANB); Keating's Gap, near Cooktown, *Blake* 20241, 1.ii.1958 (BRI, CANB); Kuranda, *Blake* 20274, 4.ii.1958 (BRI, CANB); Port Curtis District: Rosedale, *Dovey* 446, 23.ii.1933 (K, BRI); Middle Percy Island, 87 miles SE. of Mackay, *Lazarides* 5643, 27.iv.1956 (BRI, CANB); Moreton District: Yandina, *F. M. Bailey*, 1.iii.1891 (K, BRI); Maroochie, *F. M. Bailey*, 1.iii.1891 (K, BRI); Cooran, *Francis & White* iii.1916 (K, BRI); Mt. Cooroy, *White*, without date (BRI); between Buderim Mountain and Maroochydore, *White*, iv.1916 (BRI); near Buderim Mountain, *Blake* 5214, 27.ii.1934 (BRI,K); Mt. Coolum, *Blake* 13768, 16.iv.1938 (BRI); Conondale, *Goy & Smith* 647, 7.iv.1939 (BRI); Peachester, *Blake* 21208, 16.iii.1960 (BRI, CANB); Mt. Ngungun, Glasshouse Mountains, *Blake* 21225, 16.iii.1960 (BRI).

The species as occurring in Australia is extremely variable in spikelet and leaf blade length and in the size and structure of the panicle. For this reason, several of the specimens cited above warrant comment.

The collections *Goy & Smith* 647, *Blake* 5214, and *Lazarides* 4230, 5643* (Plate 2) are particularly distinctive forms in which the spikelets are barely 2·5 mm long and intermediate in size between those of *Ottochloa nodosa* and *O. gracillima* C. E. Hubbard. Furthermore, *Lazarides* 5643 and *Blake* 5214 have unusually short blades which are mostly 2·5–5 cm long and fall well within the range given in the type description of *O. gracillima*.

On the other extreme, the spikelets of *Blake* 13768, *Bailey* (Maroochie and Yandina), and *White* (between Buderim and Maroochydore) are 3·5–4 mm long and the blades on *Bailey's* specimens are up to 15·5 cm long. Also, both of *Bailey's* plants have well-developed panicles 14–22 cm long bearing 10–12 primary branches (the lowest up to 13 cm long) and several secondary branches up to 2 cm long. The specimens differ further from the majority of specimens examined by the semi-whorled attachment of some of the lower primary branches.

* This specimen probably represents a topotype of *Ichnanthus oblongus* for it is not readily ascertained on which particular island of the Percy Islands group Cunningham obtained his plant.

Generally, Australian specimens as represented by the material cited have glabrous spikelets. However, the glumes and lower lemma of *Blake* 20241 are sparsely ciliate on some margins with very slender, short, tubercle-based hairs. This condition is indicated also on *Francis & White's* specimen from Coran and *Dovey* 446 on which the lower lemmas of some spikelets are sparsely tubercled.

In Australia, *Ottochloa nodosa* is known from disjunct localities along the eastern coast of Queensland (Fig. 2). It is associated commonly with rain-forest communities and tends to favour moist shady situations.

Fig. 2.—Distribution of species.

Ottochloa gracillima C. E. Hubbard, Kew Bull. **1934**: 445 (1934).

Holotype.—Queensland: Moreton District: Moggill, *Hubbard* 8600, 3.iv.1931 (K) (isotypes in BRI, MEL, CANB).

Only the diagnostic characters warrant mention here as anything more would be merely repetition of the type description.

Very slender decumbent perennial, the flowering culms often less than 15 cm high. *Culms* multi-noded, usually with 1 or 2 longitudinal rows of soft, tubercle-based hairs, otherwise glabrous or almost so; internodes mostly less than 2·5 cm long, rarely exceeding 3·75 cm. *Leaf sheaths* glabrous or sparsely hispid particularly towards the ligule with tubercle-based hairs, ciliate on one margin, the other glabrous

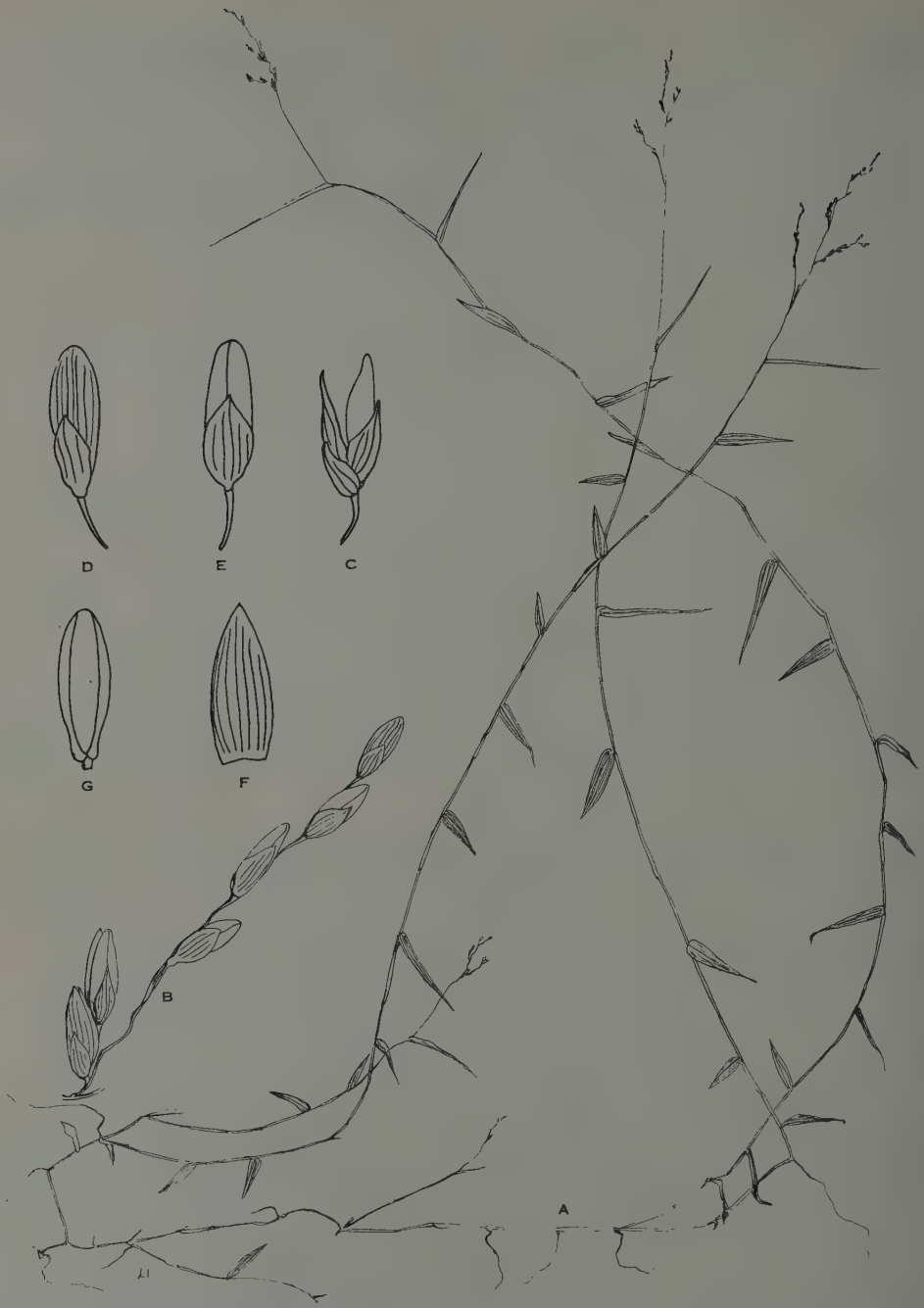
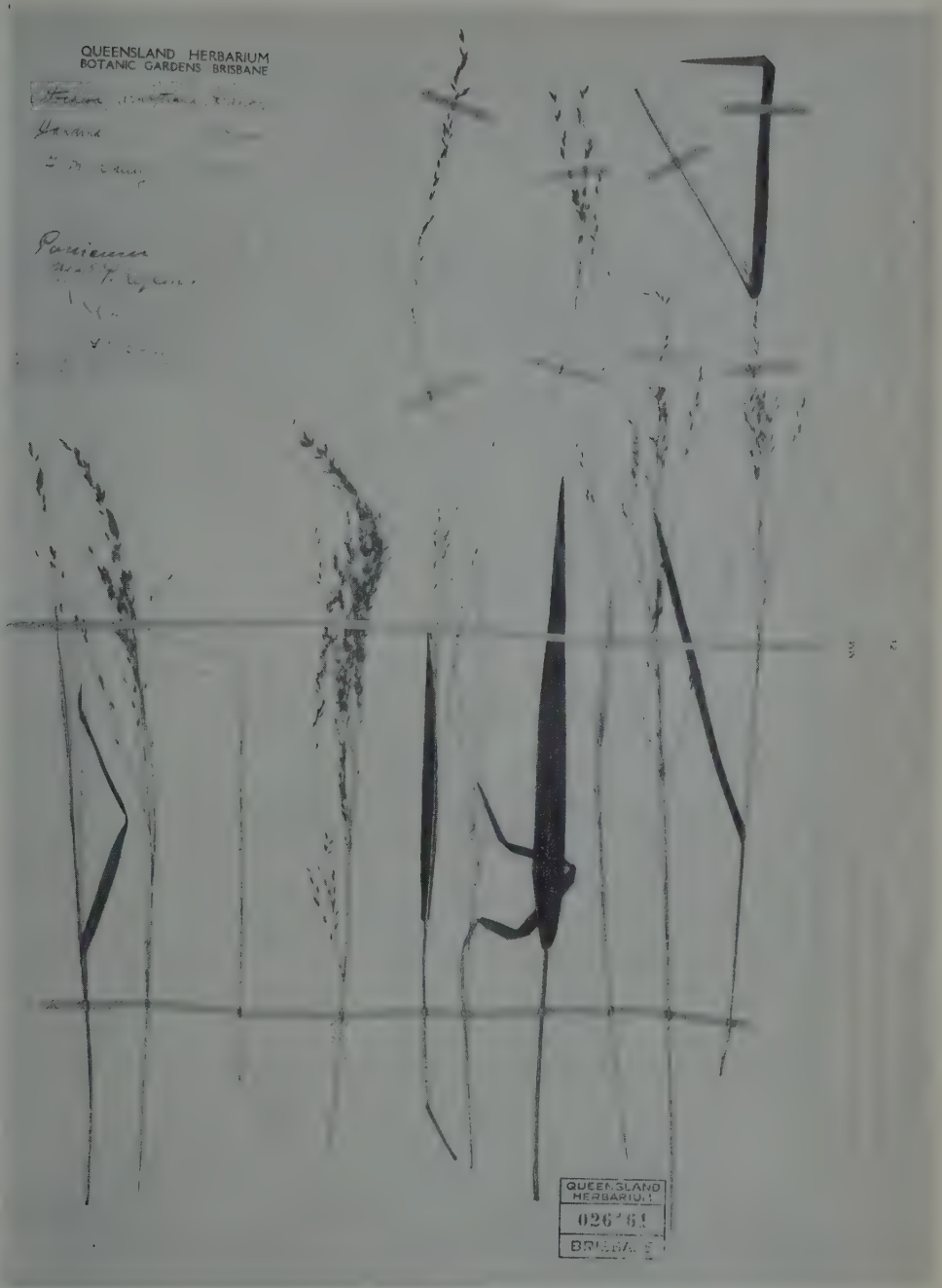
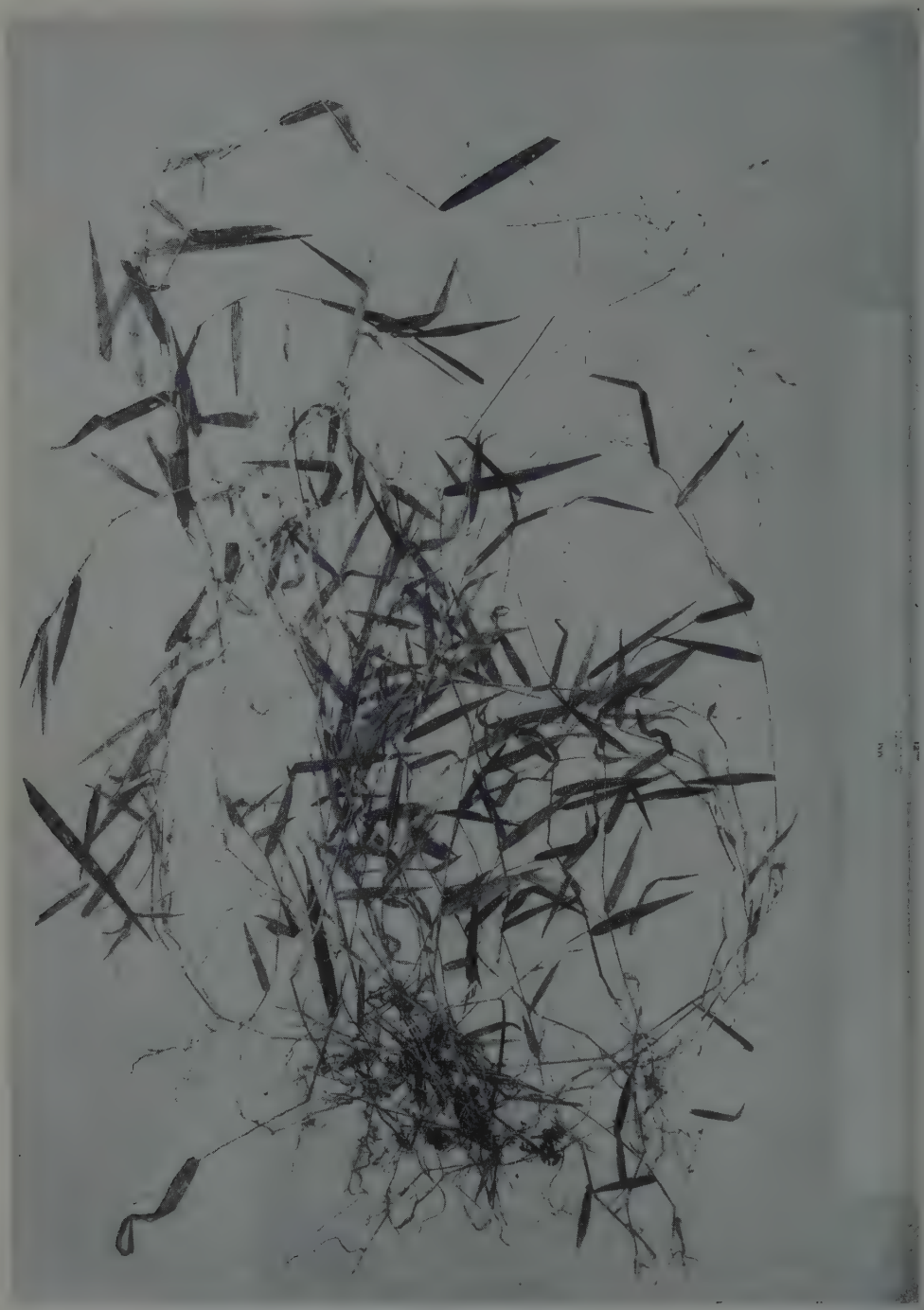


Fig. 3.—*Ottochloa gracillima* C. E. Hubbard from isotype. *A*, plant. $\times\frac{1}{2}$. *B*, panicle branch. $\times 5$. *C*, spikelet (side view). $\times 10$. *D*, spikelet (showing lower glume and lower lemma). $\times 10$. *E*, spikelet (showing upper glume and floret). $\times 10$. *F*, lower lemma. $\times 10$. *G*, floret (front view). $\times 10$.



Ottochloa nodosa (Kunth) Dandy. *Bailey's* specimen from Yandina, which matches the type of *Panicum aequabile* Domin.



Ottochloa nodosa (Kunth) Dandy. *Lazarides* 5643, suspected topotype of *Ichnanthus oblongus* Hughes.

or ciliate upwards. *Blades* usually less than 3·5 cm long and 4 mm wide. *Panicle* usually 3–5 cm long with 2–4 distant solitary primary branches. *Spikelets* mostly 1·75–2 mm long. (Fig. 3.)

The following collections are supplementary to those cited with the type description. The distribution of the species as shown in Figure 2, however, is based also on several paratypes borrowed from the Brisbane herbarium.

QUEENSLAND: Port Curtis District: Middle Percy Island, 87 miles SE. of Mackay, *Lazarides* 5645 A, 27.iv.1956 (CANB); Wide Bay District: Near Bundaberg, *Blake* 11325, 27.iv.1936 (BRI); Splitters Creek, near Bundaberg, *Goy & Smith* 600, 29.xii.1938 (BRI); Moreton District: Buderim Mountain, *Blake* 5194, 26.ii.1934 (BRI); Mt. Cotton, *Everist* 979, 7.ii.1935 (BRI); same locality, *Everist* 1002, 8.ii.1935 (BRI); Back Creek, near Canungra, *Blake* 12900, 28.iii.1937 (BRI); Brisbane, *Boorman*, iv.1899 (NSW); Mt. Gravatt, near Brisbane, *Smith* 326, 15.ii.1938 (BRI); Mt. Cootha, near Brisbane, *Smith* 497, 13.iii.1938 (BRI); Coolum, *Blake* 13767, 16.iv.1938 (BRI); Beerburum, *Burbidge* 2833, 18.iv.1948 (CANB); Mt. Ngungun, Glasshouse Mountains, *Blake* 21226, 16.iii.1960 (BRI, CANB). NEW SOUTH WALES: North Coast: Murwillumbah, *Cleland*, 4.iv.1916, N.S.W. No. 53970 (NSW); Central Coast: Erina Creek, Gosford, *Blakely*, iv.1925, N.S.W. No. 53971 (NSW).

The above specimens, with the exception of *Lazarides* 5645A, are fairly uniform. However, the remaining collection, which represents the most northerly known record and the only one from an insular locality, is a rather stout plant approaching some forms of *O. nodosa* in vegetative characters. Also, the panicles are very well developed, being up to 11 cm long with 5–8 primary branches (the lowest up to 5 cm long) and several divergent secondary branches. Nevertheless, the spikelets are distinctly those of *O. gracillima*.

Ottochloa gracillima occurs in coastal areas of southern Queensland and northern New South Wales (Fig. 2). Like *O. nodosa*, it is found commonly in shady habitats but is more often associated with open *Eucalyptus* forests than rain-forests. In the field, it forms characteristic dense mat-like colonies.

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